Early post operative serum growth hormone levels as a predictor of outcome after surgery for growth hormone (GH) secreting pituitary adenoma.

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DEPARTMENT OF NEUROLOGICAL SCIENCES
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VELLORE

CERTIFICATE

This is to certify that the dissertation titled "Early post operative serum growth hormone levels as a predictor of outcome after surgery for growth hormone (GH) secreting pituitary adenoma" is the bonafide original work of Dr. Pratheesh. R. submitted in partial fulfillment of the rules and regulations, for Branch-II M.Ch. Neurosurgery, Part-III examination of the Tamil Nadu Dr. M.G.R. Medical University to be held in February 2009.

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AIM

To evaluate the prognostic value of early post operative GH measurements with regards to remission of the disease after surgery for GH secreting pituitary adenomas.

INTRODUCTION

Anatomy and physiology

The pituitary (Figure 1) is a bean-shaped gland located at the base of the brain in the midline. It measures 0.6 cm x 0.9 cm x 1.3 cm and an average gland weighs 0.6 grams. The gland lies within the bony sella turcica. The pituitary is composed of two anatomically and functionally distinct parts: the neurohypophysis and the adenohypophysis.

The neurohypophysis is composed of the infundibulum, the pituitary stalk, and the pars nervosa of the pituitary. The cell types of the neurohypophysis include pituicytes, which are modified glial cells, and the axonal processes of neurons whose cell bodies are located in the hypothalamus. The neurohypophysis stores and releases the hypothalamic hormones oxytocin and vasopressin.

The adenohypophysis is of ectodermal origin, embryologically derived from Rathke's pouch. It has three regions, the pars distalis or anterior lobe, the pars intermedia or intermediate lobe and the pars tuberalis, an extension of epithelium that wraps around the infundibulum of the pituitary stalk.

The adenohypophysis is composed of acini that contain the specialized cell types, all of which have their own unique hormonal function and characteristics. The molecular

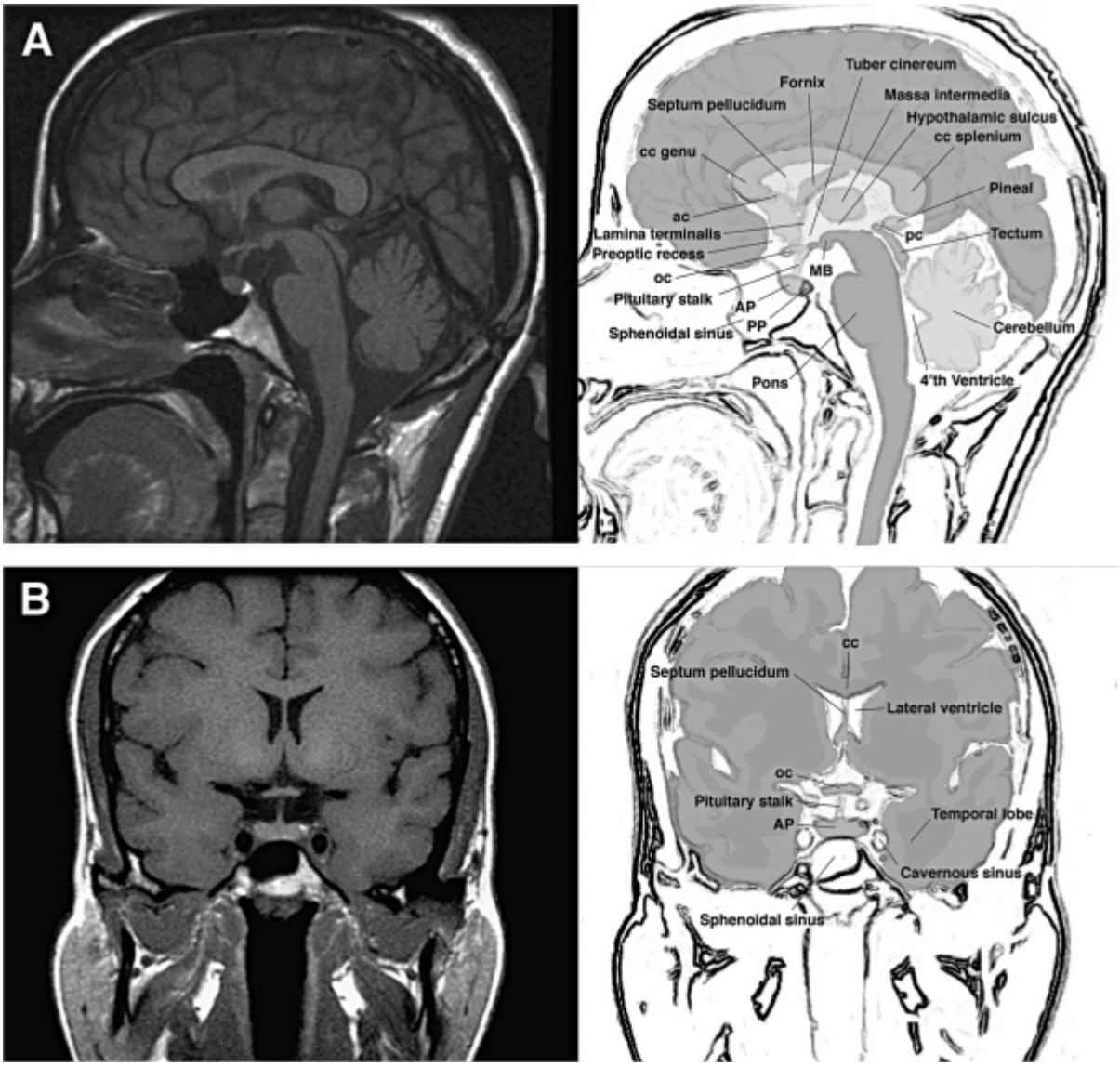


Figure1. Normal anatomy of the human hypothalamic-pituitary unit in sagittal (A) and coronal planes (B).

factors that determine hormone production are transcription factors that target specific hormone genes. Somatotrophs, lactotrophs, mammosomatotrophs, and thyrotrophs all derive from growth hormone (GH)-producing precursors.

GH-producing somatotrophs are located in the lateral wings of the anterior pituitary and account for approximately 50% of the cell population. By light microscopy they are strongly acidophilic cells with centrally located nuclei and diffuse cytoplasmic positivity for GH. Mammosomatroph cells expressing both prolactin and GH arise from the acidophilic stem cell and immunostain mainly for prolactin.

The pituitary receives its vascular supply from the superior, middle, and inferior hypophyseal arteries, all of which originate from the internal carotid arteries.

Growth Hormone and Insulin-like Growth Factor-1

The GH molecule, a single-chain polypeptide hormone consisting of 191 amino acids, is synthesized, stored, and secreted by somatotroph cells and the human GH genome locus is located on the long arm of human chromosome 17q22-24. GH is under regulation of GHRH (growth hormone releasing hormone) and SRIF (somatotropin release inhibiting factor) also known as Somatostatin, from the hypothalamus. The half-life of GH is short, 25 – 30 minutes, long enough to enable its uptake by the liver where it induces the production of insulin-like growth factor (IGF-1) known in older literature as somatomedin-C. GH does have direct effects on receptors on target cells such as

adipocytes where it induces the break down of triglycerides and suppresses the reuptake and accumulation of lipids. GH secretion is further regulated by its target growth factor, IGF-I, which participates in a hypothalamic pituitary peripheral regulatory feedback system. GH stimulates IGF-I, which exerts a negative-feedback effect on the hypothalamus and pituitary. IGF-I stimulates hypothalamic SRIF release and inhibits pituitary GH gene transcription and secretion.

IGF-1 is a trophic factor and mediates most effects of GH, through binding with IGF receptors that exist in almost every cell in the human body, particularly in muscle, cartilage, bone, liver, kidney, nerves, skin and lungs.

Acromegaly

In 1886, Pierre Marie (1) published the first clinical description of disordered somatic growth and proportion and proposed the name acromegaly. Cushing, Davidoff, and Bailey (2, 3) documented the clinicopathologic features of acromegaly and demonstrated clinical remission of soft tissue signs after adenoma resection. The prevalence of acromegaly is estimated to range from 38 to 69 cases/million, and the annual incidence of new patients is 3 or 4 per million (4). Acromegaly is caused by pituitary tumors secreting GH or very rarely by extrapituitary disorders. GH secreting pituitary adenomas constitute 10% of all pituitary tumors (5). Regardless of the etiology, the disease is characterized by elevated levels of GH and IGF-I, with resultant signs and symptoms of hypersomatotrophism.

Feedback loops involve both GH and IGF-1 that have inhibitory autocrine effects on the pituitary and hypothalamus. In acromegaly, GH hypersecretion from pituitary adenomas and rarely from ectopic sources in some malignant tumors continues unchecked since these inhibitory feedback loops are non-functional.

More than 95% of patients with acromegaly harbor a GH-secreting pituitary adenoma. Extrapituitary acromegaly accounts for the remaining 5% (6). Pure GH-cell adenomas contain either densely or sparsely staining cytoplasmic GH granules, and these two variants are either slow growing (densely granulated) or rapidly growing (sparsely granulated)(7). The former arise insidiously and manifest during or after middle age; the latter arise in younger subjects with more florid disease. Mixed GH-cell and PRL-cell

adenomas are composed of distinct somatotrophs expressing GH and lactotrophs expressing PRL. Monomorphous acidophil stem cell adenomas arise from the common GH and PRL stem cell and also often contain giant mitochondria and misplaced GH granule exocytosis. They grow rapidly, are invasive, and manifest with predominant features of hyperprolactinemia (8).

Manifestations of acromegaly are caused by either central pressure effects of the pituitary mass or peripheral actions of excess GH and IGF-I. Central features of the expanding pituitary mass are common to all pituitary masses. In acromegaly, headache is often severe and debilitating. Local signs are especially important presenting features because a higher preponderance of macroadenomas (>65%) is encountered in acromegaly, as compared to mostly microadenomas for PRL-secreting tumors (9). The clinical features are summarized in Table 1.

Effects of hypersomatotrophism on acral and soft tissue growth, and metabolic function, occur insidiously over several years. The slow onset and elusive symptomatology often results in delayed diagnosis ranging from 6.6 to 10.2 years, with a mean delay of almost 9 years (10). However in a recent study, Natchigall et al (30) showed a much shorter mean time to diagnosis (2.5 years) than previously reported in the literature and the diagnosis was suspected by the primary care physician in 44% of the cases.

Table -1

CLINICAL FEATURES OF ACROMEGALY

LOCAL TUMOR EFFECTS
Cranial nerve palsy Headache Pituitary enlargement Visual field defects
SOMATIC EFFECTS
Acral Enlargement
Thickness of hand and feet soft tissue
Cardiovascular
Asymmetric septal hypertrophy Cardiomyopathy Congestive heart failure Hypertension Left-ventricular hypertrophy
Colon
Polyps
Musculoskeletal

Acroparesthesia Arthralgias and arthritis Carpal tunnel syndrome Gigantism Hypertrophy of frontal bones Jaw malocclusion Prognathism Proximal myopathy
Pulmonary
Narcolepsy Sleep apnea—central and obstructive Sleep disturbances
Skin
Hyperhidrosis Oiliness Skin tags
VISCEROMEGALY
Kidney Liver Prostate

Salivary gland Spleen Thyroid Tongue
ENDOCRINE AND METABOLIC EFFECTS
Carbohydrate
Diabetes mellitus Impaired glucose tolerance Insulin resistance and hyperinsulinemia
Electrolytes
Increased aldosterone Low renin
Lipids
Hypertriglyceridemia
Minerals
Hypercalciuria, increased 1,25$(OH)_2D_3$ Urinary hydroxproline
Multiple endocrine neoplasia type 1
Hyperparathyroidism

Pancreatic islet cell tumors
Reproduction
Decreased libido, impotence, low sex hormone–binding globulin Galactorrhea Menstrual abnormalities
Thyroid
Goiter Low thyroxine-binding globulin

Patients might seek care for dental, orthopedic, rheumatologic, or cardiac disorders. Only 13% of 256 patients whose acromegaly was diagnosed during a 20-year period presented with primary symptoms of altered facial appearance or enlarged extremities (11). In a review of several hundred patients presenting with acromegaly worldwide, 98% had acral enlargement, and hyperhidrosis was prominent in 70 % (12). Nachtigall et al (30) did a retrospective analysis of 100 cases of acromegaly and found that the presenting symptoms in decreasing order were acral changes (24%), followed by headaches (20%), amenorrhea (6% of total, 11% of women), dental changes (4%), carpal tunnel syndrome (4%), visual deficits (3%), sexual dysfunction (3%), arthralgias (2%), galactorrhea (2%), chest pain (2%), uncontrolled hypertension (2%), diabetes (1%), dizziness (1%), gynecomastia (1% of total, 2% of men), weakness (1%), and weight gain (1%). Characteristic features include large fleshy lips and nose, spade-like hands, frontal skull bossing, and cranial ridges. Enlarged tongue, bones, salivary glands, thyroid, heart, liver, and spleen are the effects of generalized visceromegaly. Clinically apparent hepatosplenomegaly, however, is rare. Increase in shoe, ring, or hat size is commonly reported. Progressive acral changes can lead to facial coarsening and skeletal disfigurement, especially if excess GH secretion begins prior to epiphyseal closure. These include mandibular overgrowth with prognathism, maxillary widening, teeth separation, jaw malocclusion, overbite, large nose, and coarse, oily skin with large pores. Sonorous voice deepening occurs in association with laryngeal hypertrophy and enlarged paranasal sinuses. Arthropathy occurs in 70% of the patients (13). Hyperhidrosis and malodorous skin in seen in upto 70% of patients (14). Acromegaly is associated with increased morbidity and mortality, mainly secondary to cardiovascular disease.

LITERATURE REVIEW:

Clinical, biochemical and radiological diagnosis

The diagnosis of acromegaly is usually made clinically on the history and typical external appearance. GH and IGF-1 levels are elevated in acromegaly (15). In contrast to GH levels, plasma levels of IGF-I are more stable, and an elevated IGF-I level in a patient with appropriate clinical suspicion is almost always indicative of acromegaly(16). For accurate control comparison, the IGF-I level must be age and gender matched.

The various methods for GH assay include radioassays and immunoassays. The previous polyclonal radioassay techniques had a sensitivity of 0.5 ng/ml whereas the current immunoassays have sensitivity as low as 0.01 ng/ml. Freda at al (55) showed that immunoradiometric assays are superior to radioimmunoassays in distinguishing between patients with active disease and healthy subjects. Arafat et al (18) compared three different immunoassays [Immulite (Diagnostic Products Corp., Los Angeles, CA), Nichols (Nichols Institute Diagnostika GmbH, Bad Vilbel, Germany), and Diagnostic Systems Laboratories (Sinsheim, Germany)] tested on 46 acromegaly patients and 213 healthy subjects. They concluded from the study that nadir GH levels are assay, gender, age, and BMI specific, indicating the need of individual cutoff limits for each assay. Using cutoff limits of 1 ug/liter (Immulite) and 0.5ug/liter (Nichols) identified 95% of patients with active disease and 78–80% of patients in remission.

When growth hormone is measured in healthy persons with the use of standard assays, the level is usually undetectable (<0.2 μg per liter throughout most of the day), but there are approximately 10 intermittent pulses of growth hormone per 24 hours, most often at night, when the level can be as high as 30 μg per liter(19). In normal individuals GH suppression occurs after a glucose load. Improved assays with increased sensitivity have suggested that serum GH levels should normally be suppressed following an oral glucose load to less than 0.057 ng/mL in men and less than 0.71 ng/mL in women (20). Freda et al (21) showed that in normal subjects the nadir GH level after a glucose load is less than 0.14 ng/ml. The exact mechanism is not known but it is considered to be due to somatostatin release in response to glucose load. In acromegaly the basal GH levels are elevated and there is failure of GH suppression after an oral glucose load. This response is due to impaired somatostatin response or tumoral resistance to suppression by somatostatin (22). Post glucose suppression GH levels increase in one third of patients, remain unchanged in one third, or fall modestly in one third (23, 24). The following are the criteria defining diagnosis of acromegaly (25) as proposed by the consensus statement in 2000.

Random GH< 0.4 ug/l and normal IGF-1, excludes acromegaly

GH nadir during OGTT <1 ug/l and normal IGF-1 excludes acromegaly.

Following a clinical and biochemical diagnosis of acromegaly, magnetic resonance imaging studies confirm the presence of a pituitary adenoma that is then classified as micro- or macro when they are <10 or ≥10 mm in maximum diameter. MRI (26) is excellent in determining tumor invasion into the sphenoid sinus or cavernous sinuses and encasement of the internal carotid arteries all of which are factors that limit the extent of resection.

PATHOLOGY AND CLINICAL CORRELATION

The classification of GH adenomas into sparsely granulated (SG) and densely granulated (DG) types has been discussed earlier. Yamada et al (27) compared the clinical and endocrinological characteristics, neuroimaging findings, surgical outcome, and conventional histological findings (including immunohistochemistry) with the electron microscopic appearance of 31 growth hormone (GH)-producing adenomas. Sparsely granulated adenomas were found to more frequently affect younger women and also were more likely to be macroadenomas and invasive. The authors did not find any significant difference in the basal serum GH and insulin-like growth factor I levels. Light microscopy showed that densely granulated adenomas were mainly acidophilic and were immunopositive not only for GH but also for prolactin , the beta subunit of thyroid-stimulating hormone and the alpha subunit of glycoprotein hormone, whereas SG adenomas were almost all chromophobic and only revealed immunopositivity for GH. Bhayana et al (28) concluded from their study that densely granulated somatotroph adenomas were more responsive to octreotide treatment.

Obari et al (29) in a study on 104 GH cell adenomas found that sparsely granulated variant was associated with younger age, higher frequency of macroadenomas and were more invasive.

MORTALITY RATES IN ACROMEGALY

Patients with biochemically uncontrolled acromegaly perform significantly worse on health related quality of life questionnaires than those in remission or discordant remission status (31).

CARDIAC DISEASE

Symptomatic cardiac disease is present in about 20% of patients and is a major cause of morbidity and mortality (32). Hypertension is present in about 50% of patients with active acromegaly, and half of these have evidence of left ventricular dysfunction. Cardiovascular disease accounts for about 60% of deaths in patients with acromegaly, and the presence of cardiovascular disease at the time of diagnosis portends high mortality rates, despite improved cardiac function after effective GH and IGF-I control.

CANCER

A compelling cause-and-effect relationship of acromegaly with cancer has not been established. A recent controlled study in 161 patients revealed no increase in colonic polyp incidence in acromegaly (33). Analysis of nine retrospective reports (1956-1998) encompassing 21,470 person-years at risk, yielded no significant increased cancer incidence (34). Orme et al (35) showed that mortality from colon cancer is largely related

to GH levels, rather than enhanced incidence of the disease in acromegaly. Cardiovascular disease, respiratory disorders, diabetes, and malignancy account for enhanced (threefold) mortality in acromegaly (32, 35). In 194 patients with acromegaly, life expectancy was reduced, and cardiovascular disorders accounted for 24% of deaths, followed by respiratory (18%) and cerebrovascular disease (14%).

Diabetes mellitus, occurring in 20% of patients, was associated with 2.5 times the predicted mortality, and hypertension was present in about half of all patients. (36)

WHAT DECIDES CRITERIA FOR CURE?

Epidemiologic studies have shown that the high rates of morbidity and mortality associated with acromegaly can be greatly reduced by controlling GH levels (35-38, 40). But there is a difference of opinion about the GH levels to be attained. Some long term follow-up studies after transsphenoidal surgery and adjunctive therapy for acromegaly show that regardless of the stringency of criteria for cure (< 5 ng/ml vs. < 2 ng/ml), the mortality rate achieved for those in remission was equivalent to that in matched controls, in contrast to the 2.4 – 4.8 fold enhanced mortality in those patients with persistent disease(40) However, others argue that a random GH value of < 2 ng/ml was associated with a significantly lower mortality rate and should be regarded as an appropriate therapeutic target.(42) Holdaway et al (32) followed up 208 patients for a mean of 13 years studying the predictors of mortality with special reference to IGF-1 levels. They confirmed that patients with acromegaly continue to have increased mortality rates

despite treatment but the standard mortality rate (SMR: ratio between observed and expected number of deaths) can be normalized by achieving serum GH concentrations less than 1 to 2 micrograms/ml and by normalizing IGF-1 levels. One drawback of this study, is that the majority of patients received pituitary radiation as part of their treatment and this is likely to have contributed to the hypopituitarism seen in the treated group and may be a confounding factor in the analysis. Interestingly, Lindholm et al (43) recently showed that pituitary insufficiency after surgery for nonfunctional pituitary adenomas had no effect on mortality in men but the SMR was significantly higher in women. They speculate that the difference was likely to be due to insufficient gonadal replacements in women. The effect of radiotherapy on mortality in acromegaly was also highlighted by Ayuk et al (42) who found that even after controlling for GH levels, patients who underwent radiation had increased mortality with cerebrovascular disease being the predominant cause of death. These authors also found that younger patients may be at greater risk with persistent disease than older patients but that raised IGF-1 levels did not adequately predict mortality.

PREDICTORS OF CURE:

An ability to predict the long term outcome after surgery would help in initiating adjuvant therapy early. This can reduce the harmful effects of long term exposure to high GH levels. Both preoperative and post operative factors have been identified. The preoperative determinants include tumour size, preoperative GH concentration and the experience of the surgeon.

Valdemarsson et al (99) looked at the role of intraoperative GH levels in predicting long term of pituitary surgery. Venous blood samples for GH were drawn at the beginning of the operation and then intraoperatively, when the surgeon considered the tumour to have been removed (= 0min); and then 10, 20, 30, 40, 60, 90, 120 and 180min. To calculate a mean early postoperative GH level 5-7 blood samples were drawn on the 7th post operative day after surgery. The $t_{1/2}$ GH (Figure 2) was calculated from the intraoperative blood samples drawn at 0, 10, 20, 30 and 40min. Growth hormone elimination was assumed to follow first order kinetics and the slope of the graph between time and serum GH level was used to calculate the half life of GH. A calculated GH half life of <31 min had a positive predictive value of 72%. However they found that mean post operative GH at one week had a predictive value of 100% when <2.6 ng/ml. Another paper published by the same group (100) showed that a mean serum GH level of < 2 ng/ml sampled within the first postoperative week was found to have 77% specificity and a predictive value at 97% for a satisfactory effect with regard to the outcome of the operation as evaluated 3 months later (the definition of cure being post glucose suppression GH level of < 2 ng/ml or a normalized IGF-1).

Multiple studies have shown that microadenomas have a much better remission rate as compared to macroadenomas. The remission rates using the current stringent criteria for microadenomas range from 75-100% whereas for macroadenomas the range is 21-78% (48, 51, 53, 56, 61, 62, and 64).

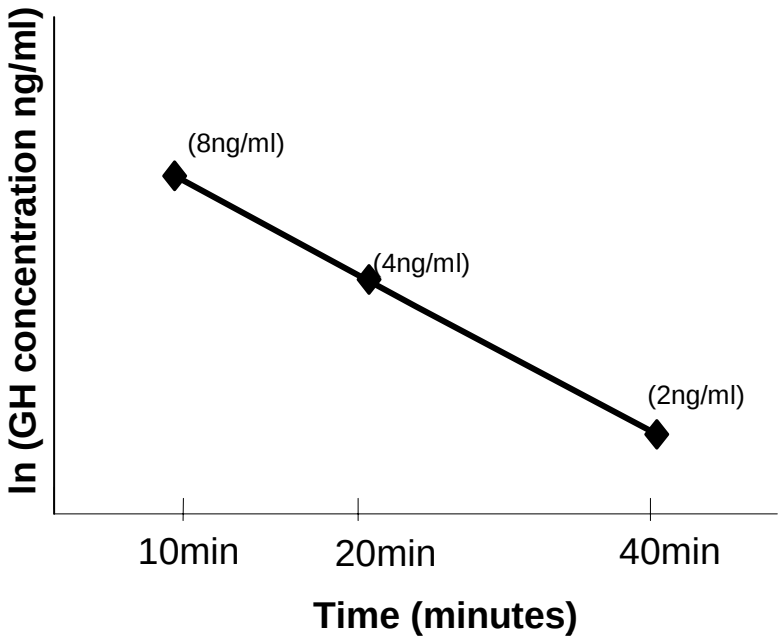


Figure 2: Graph showing log (serial intraoperative GH measurements) versus time

Similarly Osman et al (94) found that a minimum GH of < or = 2 mU/l during an OGTT (oral glucose tolerance test) was achieved in 67.4% of patients with intrasellar tumours, compared with 27.3% with extrasellar tumours.

Kaltsas et al (95) showed that patients who achieved remission had significantly lower preoperative mean GH levels than patients who were not cured (median, 31 mU/L vs. 78.5 mU/L, $P < 0.01$).

De et al (53) showed that if the preoperative GH level was < 10 ng/ml then there was 86% chance of remission. Similarly Shimon et al (39) in their study showed only a 20% chance of remission if the preoperative GH level was more than 50 ng/ml.
Shimon et al preop GH > 50 ng/ml only 20% chance for remission.

Ahmed at al (48) and Lissett et al (59) demonstrated significant increase in the remission rates over time with increase in the surgeon's experience.

Bourdelot et al (96) listed the following predictors for poor outcome. High IGF-I concentration before surgery, young age, high basal GH concentration before surgery and high nadir GH/OGTT before surgery. The MRI predictors of poor outcome were: adenoma greater than 15mm in diameter, infrasellar extension, suprasellar extension and invasive adenoma.

Studies that looked at electron microscopy features of growth hormone adenomas found that sparsely granulated adenomas were more invasive and had lower surgical cure rates (27, 29). Densely granulated growth hormone adenomas were also found to be more responsive to octreotide treatment (28).

The post operative factors which have been evaluated, include growth hormone levels and IGF-1 levels for assessing disease response to surgery and predicting long term remission. Most studies have used basal/random GH values as an indicator of disease activity and the response to surgery (40, 63, 67, 94, 97, and 98).

Kaltsas et al (95) evaluated 67 patients with acromegaly. At 7 days following transsphenoidal surgery, GH secretion was assessed by calculating the mean serum GH from a five-point GH "day curve," samples being collected at 0830 h, 1100 h, 1300 h, 1700 h, and 1900 h. The authors took an early postoperative GH level of < 5ng/ml as "safe". Relying on a single GH measurement alone, 9 of the 23 patients with a single postoperative mean GH level less than 5 mU/L obtained at least one GH value of more than 5 mU/L (false positive rate, 28%) and 8 of the patients with a postoperative mean GH value of more than 5 mU/L obtained a single GH value of less than 5 mU/L (false negative rate, 15%), thus showing that early mean post operative GH levels were more sensitive as compared to random GH level. They suggested that mean serum GH levels and single IGF-I levels, measured early in the postoperative period, are currently the best biochemical guide to the adequacy of surgery and, hence, the need for further treatment.

Takahashi et al (101) examined IGF-1 and glucose suppressed GH levels within the first postoperative month, and correlated it with cure at final follow-up (as documented by a normalized IGF-1 level). The mean follow-up period was 7 years, and they found that patients who attained both normalized IGF-1 and glucose suppressed GH < 1ng/ml, stayed cured. However, even in patients who did not meet both criteria within the first postoperative month, glucose-suppressed GH < 1.5 ng/ml, or glucose-suppressed GH < 4 ng/ml coupled with early IGF-1 normalization indicated the possibility of cure. None of the patients with an early glucose-suppressed GH > 4 ng/ml, achieved cure.

Feelders et al (102) did a prospective study with 1-yr follow-up on 17 patients who underwent transsphenoidal surgery for acromegaly. The outcome measures included OGTT at 1, 2, 3, 8, and 12 wks after transsphenoidal surgery; weekly measured GH, IGF-I and total IGF-I levels measured at 52 wks. According to the postoperative GH nadir, patients were classified into two groups using 1 ng/ml as the cutoff value. The nine patients, who were cured at one year, all had nadir GH levels in the first post operative week of 0.5 ng/ml or less. They concluded that 1 week post glucose suppressed GH level of 0.5 ng/ml or less had a high predictive value for cure. Because IGF-I showed varying patterns towards stabilization, they recommended against using it as a predictive parameter within 3 months after surgery.

TREATMENT OF GH-SECRETING PITUITARY ADENOMAS

Definition of cure or remission in acromegaly

The definition of cure in acromegaly has undergone several revisions over the years, each making the criteria more stringent with the development of more sensitive assays for GH and IGF-1. Various investigators have advocated the use of fasting GH levels, mean GH values of a series collected over 24 h, GH nadir during the first 2 hours following OGTT, or IGF-I levels to assess the outcome of treatment in acromegaly(107). Although IGF-I levels correlate well with clinical activity of acromegaly (55), they have been thought insufficient alone in predicting the long-term benefits of surgery (16). This view was supported by Sheaves et al. (65), who favored a combination of a GH day profile and IGF-I levels in the follow-up of treated patients with acromegaly. Puder et al (44) have shown that in the presence of discordant nadir GH and IGF-1 values, IGF-1 was a better predictor of disease control than random/post suppressed GH values. The study was based on insulin sensitivity as an indicator of disease control. Insulin resistance is a very common metabolic abnormality in patients with active acromegaly and is a major contributor to the increased cardiovascular risk associated with this disease. Buchfelder et al. (105) and Stoffel-Wagner et al. (106) have demonstrated that a nadir GH level of less than 1.0 ng/ml during OGTT provides much better prognostic information than random GH measurements in determining remission of acromegaly. Most recently, Giustina et al (25) have provided good evidence that, in addition to a mean random GH level below 2.5

ng/ml, treatment goals should restore GH levels to less than 1.0 ng/ml during an OGTT and normalize age- and gender-related IGF-I levels.(Consensus statement, 2000).

When to assess for cure?

The timing of postoperative testing may also be important, and recent evidence from Kaltsas et al, (95) suggests that early assessment may be as effective as more traditional analyses in which formal testing of the GH axis is delayed until 1 month or more after surgery. They found no differences in remission rates assessed at 1–3 wk compared with more than 4 wk after surgery and propose that early measurement of IGF-I is prognostically useful. This is in contrast to others who have suggested that early postoperative analysis may be inaccurate (25). However IGF-1 as an indicator of cure is significant at 3 or more months after surgery (102).

Surgery:

The overwhelming advantage of surgery over other modalities of therapy is the prompt reduction in tumor volume and relief of chiasmal compression. Currently, surgery is still considered to be the first line treatment for acromegaly and medical/radiation therapies are considered adjuncts should surgery fail (45). Transsphenoidal surgery is the procedure of choice and craniotomy is very rarely indicated. In microadenomas, complete adenomectomy with preservation of normal adenohypophysis is recommended. In macroadenomas, radical surgery is the goal, recognizing that a peripherally located tumor capsule represents normal adenohypophysis (103). In patients who fail to achieve

surgical cure, reoperation for surgically accessible residual or recurrent tumor seen on MRI should be considered (45).

Results of surgery

Cure rates: Several recent series have reported on the results of transsphenoidal surgery. Symptomatic relief occurs in nearly 95% of cases (68). Cure rates with the stringent criteria of nadir GH levels < 1.0 ng/ml during a GTT vary from 35.5% to 84.4% (see table 2). With less strict criteria the remission rates are higher: 76% when < 5 ng/ml GH levels were used (46) to 94% when < 10 ng/ml was the criterion for cure (47).

Table 2. compares the results of transsphenoidal surgery for acromegaly in literature.

Table:2

PRIMARY TRANSSPHENOIDAL SURGERY FOR GH-SECRETING PITUITARY ADENOMA

Series	Number of Cases	Total Cure Rate (%)	Microadenomas (%)	Macroadenomas (%	Definition of Cure
Davis et al, 1993(52)	174	52	N/A	N/A	GH <2 ng/ml (<4 mU/I; basal or OGTT)
Sheaves et al, 1996(65)	100	42	61	23	GH <2.5 ng/ml (<5 mU/I)
Swearingen et al, 1998(66)	162	57	91	48	Normal IGF-I levels
Lissett et al, 1998(59)	73	18	39	12	GH <5 ng/ml after OGTT

Series	Number of Cases	Total Cure Rate (%)	Microadenomas (%)	Macroadenomas (%	Definition of Cure
Abosch et al, 1998 (40)	254	76	75	71	GH <5 ng/ml (<10 mU/I)
Ahmed et al, 1999(48)	97	—	90	56	Basal GH ≤2.5 ng/ml (<5 mU/I), OGTT GH <1 ng/ml (<2 mU/I), normal IGF-I levels
Biermasz et al, 2000(49)	59	41	NA	NA	Basal GH ≤ 2.5 ng/ml (<5 mU/I)

Series	Number of Cases	Total Cure Rate (%)	Microadenomas (%)	Macroadenomas (%	Definition of Cure
Laws et al, 2000(58)	117	67	87	51	Basal GH <2.5 ng/ml (<5 mU/I), OGTT GH <1 ng/ml (<2 mU/I), normal IGF-levels)
Fahlbusch, 2001(41)	490	56	78	50	Basal GH <5 ng/ml (<10 mU/I), OGTT GH <2 ng/ml (<4 mU/I), normal IGF-I levels
Kreutzer et al, 2001(57)	57	70.2	NA	NA	OGTT GH <1 ng/ml (<2 mU/I)

Series	Number of Cases	Total Cure Rate (%)	Microadenomas (%)	Macroadenomas (%	Definition of Cure
De et al, 2003(53)	90	63	79	56	OGTT GH <1 ng/ml (<2 mU/I), normal IGF-levels
Minniti et al, 2003(61)	92	55	80	50	OGTT GH <1 ng/ml (<2 mU/I), normal IGF-levels
De et al, 2003(53)	90	63	79	56	OGTT GH <1 ng/ml (<2 mU/I), normal IGF-levels
Nomikos et al, 2005(62)	668	57.3	75.3	50.3	OGTT GH <1 ng/ml (<2 mU/I), normal IGF-levels

Series	Number of Cases	Total Cure Rate (%)	Microadenomas (%)	Macroadenomas (%	Definition of Cure
Abbasioun et al, 2006(47)	104	94.2	NA	NA	Basal GH< 10 ng/ml
Boeving et al, 2006(50)	28	35.5	NA	NA	Basal GH ≤2.5 ng/ml (<5 mU/I), OGTT GH <1 ng/ml (<2 mU/I), normal IGF-I levels
Boeving et al, 2006(50)	28	35.5	NA	NA	Basal GH ≤2.5 ng/ml (<5 mU/I), OGTT GH <1 ng/ml (<2 mU/I), normal IGF-I levels

Series	Number of Cases	Total Cure Rate (%)	Microadenomas (%)	Macroadenomas (%	Definition of Cure
Santoro et al, 2007(64)	109	61	85	55	OGTT GH <1 ng/ml (<2 mU/I), normal IGF-levels
Gondim et al, 2008(56)	33	84.84	100	78.2	OGTT GH <1 ng/ml (<2 mU/I), normal IGF-levels

Many studies have assessed recurrence rates after transsphenoidal surgery for acromegaly, but these rates vary considerably, possibly because of lack of uniformity in the criteria used to define remission. It is likely that older studies overestimated the cure rates due to the lack of precise GH assays. Modern series with long-term follow-up, report recurrence rates between 1.1 and 19% (57, 40, 49, and 66). Freda et al (69) studied 110 postoperative patients with acromegaly and defined remission by normal IGF-1 levels. The 76 patients who were in remission were divided into two groups – Group 1 were those with nadir post-suppression GH levels < 0.14 ng/ml and Group II were those with nadir post-suppression levels >0.14 ng/ml. Group I patients continued to be in remission on long-term follow-up however, 5 of the 19 patients in Group II were found to have elevated IGF-1 levels 2 to 6 years after surgery and were diagnosed to have biochemical recurrence. They concluded that those patients in remission postoperatively who have a subtle failure of normal GH suppression have additional evidence of relatively increased GH secretion compared with those patients with normal GH suppression. They suggest that as long as IGF-1 normalization is maintained, these patients can be observed without additional therapy. However, with longitudinal follow-up, abnormal GH nadir was associated with a higher rate of disease recurrence as defined by the development of elevation in IGF-1 levels and these patients may need to be monitored more closely for recurrence.

Presurgical treatment with somatostatin analogs:

The evidence that preoperative GH level, tumor size and invasiveness contributed to outcome prompted the use of perioperative pharmacotherapy to enhance results of surgery (70). Colao et al (71) showed that surgical debulking helped in increasing remission rates from 12% to 76% in patients harboring invasive tumors that responded poorly to primary medical therapy, that is, somatostatin analogs, for at least 6 months before surgery. On the other hand, in a recently performed large case-controlled study (72), 143 patients who received somatostatin analogs for 3 months before surgery (Group I) were matched for tumor size and invasiveness with 143 patients who had direct surgery without pharmacotherapy (Group II). Although there was a 50% reduction in GH levels with medical therapy in 64% of cases before surgery in Group I, the overall surgical remission rate was 57% in group I and 64% in Group II. The conclusion was that pretreatment with somatostatin analogs (octreotide, lanreotide or octreotide LAR) did not provide any benefit in cure rates after surgery. However the study by Carlsen et al (73) shows better cure rates with somatostatin analog pretreatment. They randomized 61 newly diagnosed acromegalics into two groups- direct transsphenoidal surgery (n=30) or pretreatment with octreotide (n=31) 20mg intramuscularly every 28th day for 6 months before transsphenoidal surgery. Cure was evaluated 3 months postoperatively primarily by normalized IGF-I levels. 14 of 31(45%) pretreated patients vs. seven of 30 (23%) patients with direct surgery were cured. In patients with microadenomas (<10 mm), one of five (20%) pretreated vs. three of five (60%) with direct surgery were cured. In patients with macroadenomas, 13 of 26 (50%) pretreated vs. four of 25 (16%) with direct surgery were cured.

Radiotherapy

The role of radiotherapy (RT) in acromegaly is almost entirely restricted to those patients who fail previous surgery, since pituitary dysfunction and delay to remission limit its utility. As primary therapy, conventional RT is known to reduce tumor mass, GH levels and pituitary function predictably with time. Further growth is prevented in 99% of patients; GH levels progressively decrease to 50% of baseline by 2 years and 75% of baseline by 5 years. Further decrease in GH levels are seen at 10 and 15 years and 90% of those surviving at 15 years have levels < 5 ng/ml. Using more stringent criteria of cure, post suppression GH levels < 1ng/ml after a GTT, were seen in 9% of patients at 2 years, 29% at 5 years, 52% at 10 years and 77% at 15 years when radiation was used as an adjunct to surgery (74). Fifty percent of patients who underwent treatment with both radiation therapy and surgery have reported hypopituitarism, and the incidence can increase with time from exposure for up to 20 years post treatment (75, 76). Minniti et al (74) noted progressive hypopituitarism, which was present in 33% of patients at baseline and increased to 57%, 78% and in 85% of patients at 5 10 and 15 years after RT, respectively.

Fractionated conformal stereotactic radiotherapy in patients with residual or recurrent tumors, achieves tumor control and normalization of GH levels at rates comparable to conventional RT (77). Moreover, the rate of decline and normalization of GH levels are within the same reported range following conventional RT. Delayed hypopituitarism is still a problem with incidence varying from 5% to 29% (77-79) depending on the duration of follow-up.

Gamma knife radiosurgery (GKS) for acromegaly appears to show promising results but long-term follow-up is necessary to ascertain the incidence of hypopituitarism (80, 81). Witt et al (82) reviewed 20 different radiosurgery studies published between 1997 and 2002 and found that tumor control in acromegaly ranged from 68 to 100%. He also found endocrine improvement in 0 to 67%, depending on the study. The endocrine cure in the different studies ranged from 0 to 96%. Zheng et al (83) used GKS as primary therapy for acromegaly in 68 patients, which constituted 80% of their cases and had a follow up for a mean of 34 months. They do not define their criteria of cure but mention that "normalization of the hormonal levels" was achieved in 23 (40%) of 58 patients followed for 12 months, and in 96% of cases followed for more than 24 months. One patient with an invasive tumor developed bilateral 3rd nerve palsies that recovered in 6 months and another patient had reduced visual acuity one year after GKS. No details are available for incidence of hypopituitarism. Jezkova et al (84) suggest that GKS is a useful adjunct to surgery in acromegaly when treating residual tumor and recommend its use as primary therapy only when surgery is not possible. The majority of their patients (75%) had previous surgery and only 25% were treated primarily with GKS with normalization intervals shorter than in conventional RT. They used three different criteria to establish a successful outcome, but the results of the most commonly accepted criterion, post suppression GH levels < 1ng/ml in an oral GTT and normal IGF-1 levels a median interval to normalization of 66 months the percentage of patients achieving a cure at 1, 3, 5 and 8 years was 14.6%, 28.6%, 44.2% and 57.1% respectively. They noted that 32% of patients developed thyroid hormone deficiency, 14% adrenal insufficiency and 41%

gonadal deficiency. Losa et al (85) looked at stereotactic radiosurgery without concomitant GH-suppressive drugs in 83 patients with residual or recurrent GH-secreting adenomas. The rate of remission (GH level < 2.5 ng/ml) was 52.6% at 5 years. The 5-yr cumulative risk of new onset hypogonadism, hypothyroidism, or hypoadrenalism was 3.6%, 3.3% and 4.9% respectively.

Medical Therapy

Dopamine agonists, such as bromocriptine and cabergoline, reduce basal GH to < 5ng/ml in only 10-30% of cases and normalize IGF-1 levels in <10% (86). There are some reports that mixed GH-PRL tumors respond better to dopamine agonists (86). These are relatively cheap drugs and may have some role in the postoperative treatment of patients with residual disease. Somatostatin analogues are more effective than dopamine agonists and studies have convincingly shown their efficacy on hormonal levels and tumor size (87). These drugs were used as adjuvant therapy alone but have been employed as primary treatment when financial constraints do not exist.(88) In a prospective long-term follow-up study, Cozzi et al (89) treated 67 naive patients with acromegaly with octreotide LAR but 33 patients elected to undergo other forms of therapy during the study. Their outcome measures were basal GH (<2.5 ng/ml), normal IGF-1 levels and tumor shrinkage. GH and IGF-1 levels decreased mostly within 6-12 months but progressive declines resulted in hormonal endpoints in 57% of patients. Five percent of patients were unresponsive to treatment, that is, < 10% change in GH/IGF-1 levels in 6 months. Pretreatment GH levels made no difference to the outcome. Tumors shrank in 82% of patients within 6 months and continued to decrease in size.

Macroadenomas tended to show a more impressive response than microadenomas with >50% size reduction in 70% of cases, though two of three tumors that disappeared were microadenomas. Invasiveness, however was not reverted, though in a few cases those tumors that showed doubtful invasion proved to have clear borders separate from the cavernous sinus wall with treatment. These findings are supported by more recent studies (90) that have shown significant tumor size reduction and GH normalization at 6 months; though the results were worse for macroadenomas with only 45% of patients achieving GH levels < 2.5 ng/ml. At the present time, surgery and medical therapy complement each other in the overall management of acromegaly. Recent data points towards a role for surgical debulking in improving response to somatostatin agonists in tumors that are only partially sensitive to these drugs (71, 91).

The disadvantages of octreotide are that it tends to produce gastrointestinal side effects and predisposes to the development of gallstones. While it requires thrice-daily subcutaneous injections, long acting formulations of somatostatin analogs (octreotide LAR and lanreotide) are as effective but some patients may not respond to these drugs optimally or become intolerant.

Pegvisomant, the first recombinant GH-receptor antagonist, does not inhibit the production of GH but binds at peripheral GH receptors and prevents IGF-1 production. Therefore its action is independent of tumor characteristics such as size and invasiveness and it effectively normalizes IGF-1 levels in 70% of patients by 12 months (92). Since pegvisomant is detected in conventional GH assays, it may result in falsely high estimation of serum GH concentrations; therefore its efficacy can be monitored only by IGF-1 levels and not by GH levels. Furthermore, some tumors can in fact grow while on

treatment with this drug therefore patients need to be monitored for visual deterioration and tumor growth on MRI. The efficacy of pegvisomant is dose dependent and IGF-1 levels have been normalized in those patients who were surgical failures or who were resistant to or intolerant of somatostatin analogs (93).

Table 3. compares the advantages and disadvantages of the various treatment modalities for acromegaly.

Table 3- Various treatment modalities in acromegaly.

Characteristic	Surgery	Radiation Therapy	SRL	GHR Antagonist	Dopamine Agonist
Advantages					
Mode	Transsphenoidal resection	Noninvasive	Monthly injection	Daily injection increases	Oral
GH <2.5 μg/L	Macros <50%	35% in 10 yr	80%		<15%
	Micros >80%				
IGF-I normalized		<30%	>70%	>90%	<15%
Onset	Rapid	Slow (years)	Rapid	Rapid	Slow (weeks)
Patient compliance	Onetime consent	Good	Must be sustained	Must be sustained	Good
Tumor mass	Debulked or resected	Ablated	Growth constrained or shrinks <50%	Unknown	Unchanged
Disadvantages					
Cost	One time	One time	Ongoing	Ongoing	Ongoing

Characteristic	Surgery	Radiation Therapy	SRL	GHR Antagonist	Dopamine Agonist
Hypopituitarism	<10%	>50%	None	Very low	None
				IGF-I if overtreated	
Other	Tumor persistence or recurrence 6%	Local nerve damage 2nd brain tumor	Gallstones 20%	Elevated liver enzymes (rare)	Nausea □30%
	Diabetes insipidus 3%	Visual and CNS disorders, 2% cerebrovascular risk	Nausea, diarrhea		Sinusitis
	Local complications 5%				High dose required

MATERIALS AND METHODS:

Study design:

Retrospective analysis of a prospectively maintained database.

Study population:

Patients with acromegaly who underwent transsphenoidal surgery at the Department of Neurosurgery from the time period May 2001 till January 2007.

The patient information was recorded in a proforma (Appendix-1) which included patient details, pre- operative, intraoperative and post operative data along with details of follow-up.

All patients had a complete hormonal evaluation and magnetic resonance imaging of the brain.

Tumors were classified as microadenomas when they were <10 mm and macroadenomas were those that were 10 mm or larger. Wilson's modification (104) of Hardy's staging system graded the macroadenomas into A, B, C, D or E. Invasion was defined as involvement of the cavernous sinuses, sphenoid sinus or intraoperative evidence of dural invasion.

Criteria for preoperative diagnosis of acromegaly:

1. Clinical evaluation
2. Elevated basal serum growth hormone levels of > 2.5 ng/ml
3. Failure of growth hormone level to suppress below < 2 ng/ml following a glucose load

(100 gm)

4. Histopathology reported as Growth hormone secreting pituitary adenoma.

Early post operative serum growth hormone levels were measured on the first morning after surgery (basal level) and seventh day (post suppression with 100 mg oral glucose) following transsphenoidal surgery.

The GH hormone assay was done on Immulite-2000 using a Diagnostic Products Corporation kit. It is a solid phase, 2-site chemiluminescent immunometric assay, using murine monoclonal anti-HGH antibody and rabbit polyclonal anti-HGH antibody, conjugated to alkaline phosphatase. The sensitivity of the assay is 0.01 ng/ml with linearity upto 40 ng/ml.

Histopathological evaluation included immunostaining for cytokeratin to classify the growth hormone secreting adenomas into sparsely/densely granulated adenomas. The detailed histopathological methodology is enumerated in Appendix-2.

The patients were evaluated at follow-up with glucose suppressed serum growth hormone level measurements and IGF-1 after July 2004. The IGF-1 assay was done on Immulite-2000 using a Diagnostic Products Corporation kit. It is a solid phase, 2-site chemiluminescent immunometric assay, using murine monoclonal anti-IGF-1 antibody and rabbit polyclonal anti-IGF-1 antibody, conjugated to alkaline phosphatase. The sensitivity of the assay is 20 ng/ml. The reference adult ranges are given in Appendix-3.

Criteria for remission:

1. Glucose suppressed serum growth hormone level of less than 1 ng/ml.

2. IgF-1 level in the normal range for age (IgF-1 levels were available and standardized at our institution only after July 2004).

Analysis:

The patients who had follow-up were included in the analysis and the following predictors of cure were evaluated – duration of symptoms, preoperative GH levels, size of tumour, Hardy's grade, invasiveness, extent of excision, electron microscopy findings on histopathology and the main focus being on early post operative GH levels.

RESULTS:

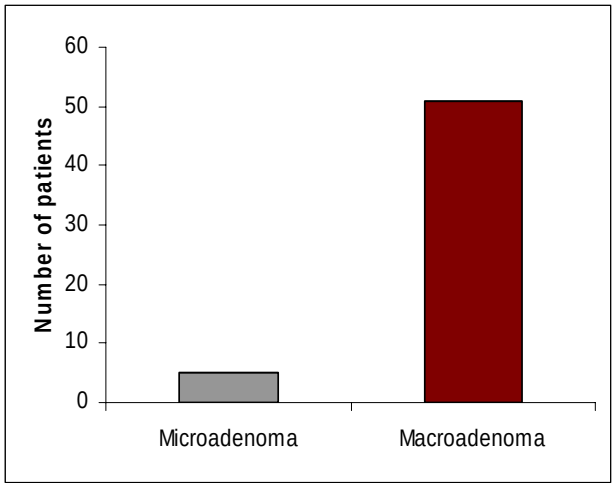
A total of 72 patients underwent transsphenoidal surgery for acromegaly between May 2001 till January 2007. Their ages ranged from 16 to 75 years (mean 37.5) and there were 36 women and 36 men. Three patients did not have clinical features of acromegaly, the diagnosis being made on biochemical and immunohistochemical basis. In the remaining 69 patients, the duration of acromegaly ranged from 3 months to 15 years (mean 52 months). Preoperative vision was affected in 25 cases (35%). Twenty five (35%) patients had diabetes, 27 (37%) had hypertension (HT) and 13 (18%) had both HT and diabetes. There were 6 microadenomas and the remaining 66 were larger than 10 mm. Of the macroadenomas, 16 were Hardy's stage A, 16 Stage B, 23 Stage C, 5 Stage D and 6 Stage E. Mixed GH-PRL tumors were seen in 16 patients. Immunostaining for cytokeratin was available for 67 out of the 72 patients and in 52 of the 56 patients with follow-up. Based on the immunostaining for cytokeratin, tumours were classified into sparsely or densely granulated somatotroph adenomas. In the 52 patients, 25 patients had sparsely granulated growth hormone adenomas and the rest were densely granulated.

The mean preoperative GH level was 34 ng/ml. Based on the preoperative basal GH levels, the patients were divided into two groups. The GH levels were <= 34 in 42 cases; > 34 in 30 cases. The GH levels in the 6 microadenomas were <= 34 in 5 cases and > 34 in 1 patient. Twenty seven cases out of the 72 were found to be invasive either on the MRI or at surgery. In the 56 cases with follow-up the proportion of invasive tumours was 20/56 (35.7%).

Follow-up was available in 56 patients (77.77%) ranging from 3 months to 65 months (median 21 months).

5 out of the 56 patients were microadenomas and the remainder were macroadenomas. (Figure 3)

Figure 3



Bar diagram showing distribution of microadenomas and macroadenomas.

Early post operative GH levels as a predictor of cure:

The 1st post operative day GH levels were available in 45 patients and the 7th post operative day GH levels were available in 40 patients. These values were evaluated to find out the early post operative GH level which would serve as a good indicator of cure. Initially a ROC (receiver operating characteristic) curve was used to plot the values and find out the GH level with the highest sensitivity and specificity. However, during the analysis it was found the area under the ROC curve was not significant because of the small number of patients. Hence ROC could not be used to find the specific GH level which was a good indicator of cure. Most of the adenomas which had been cured, had early GH values below 5 ng/ml. Hence we calculated the specificity and sensitivity of values from 1 – 5 ng/ml separately for predicting a cure. Statistical analysis was done using the 2 x 2 table. It was found that for both the first post operative day and the seventh post operative day, a value of 2 ng/ml yielded the greatest specificity and sensitivity.

1st post operative day GH level (Figure 4) of > 2 ng/ml had a negative predictive value (NPV) of 96.4%, positive predictive value (PPV) of 76.4%, sensitivity of 92.85% and a specificity of 87.1%. The P value was <0.05.

This meant that if a patient who underwent transsphenoidal surgery for acromegaly, has a 1st postoperative day GH level of more than 2 ng/ml, it is almost certain that he won't be cured (NPV of 96.4%). Similarly if the value is less than 2 ng/ml, then he has a high chance of cure (PPV of 76.4%). That this test is significant is confirmed by the high sensitivity and specificity.

Similarly the 7th post operative day GH level (Figure 5) of > 2ng/ml had a negative predictive value of 88.36%, positive predictive value of 72.5%, sensitivity of 81.25% and specificity of 79.16%. The P value was <0.05.

Thus, similar to the 1st postoperative day value, the 7th postoperative day value of more than 2 ng/ml was associated with high chance of failure and a value of less than 2 ng/ml correlated to a high chance of cure. Here again the sensitivity and specificity were significant.

Thus, the analysis showed that early postoperative GH levels were a good predictor of cure with a high specificity and sensitivity.

Figure 4.

1st postoperative day basal GH level vs. remission

n=45

Remission	GH Level <2 ng/ml	GH Level >2 ng/ml
YES	13	1
NO	4	27

Negative predictive value 96.4%
Postive predictive value 76.4%
Sensitivity 92.85%
Specificity 87.1%
P value <0.05

Figure 5.

7th postoperative day glucose suppressed GH level vs. remission

n=40

		GH Level	
		≤2 ng/ml	**>2 ng/ml**
Remission	**YES**	13	3
	NO	5	19

Negative predictive value 86.36%
Postive predictive value 72.5%
Sensitivity 81.25%
Specificity 79.16%
P value <0.05

Other predictors:

The remission rates based on Hardy's grade: (Figure 6)

Microadenoma: 60% (3/5)

Grade A- 35.7% (5/14)

Grade B- 54.5% (6/11)

Grade C- 47% (8/17)

Grade D- 25% (1/4)

Grade E- 0% (0/6)

The p-value on a Chi square test for determining the significance of size was 0.45 with lower Hardy's grade (A,B,C) having an odd's ratio of 6.61 for cure.

Remission with relation to preoperative random GH value: (Figure 7)

The patients were divided into 2 groups based on the mean preoperative GH level.

19 of the 33 patients with GH level < 34 ng/ml were in remission (57.6%) whereas only 4 out of the 23 patients with GH level > 34 ng/ml were in remission (17.4%). The p-value on a Chi square test was 0.0002 which was significant.

Figure 6

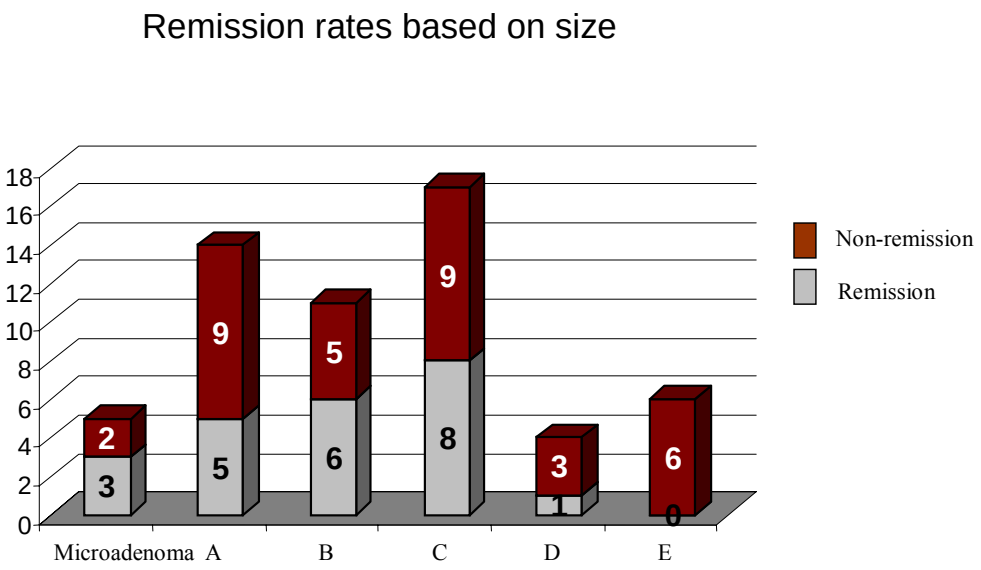
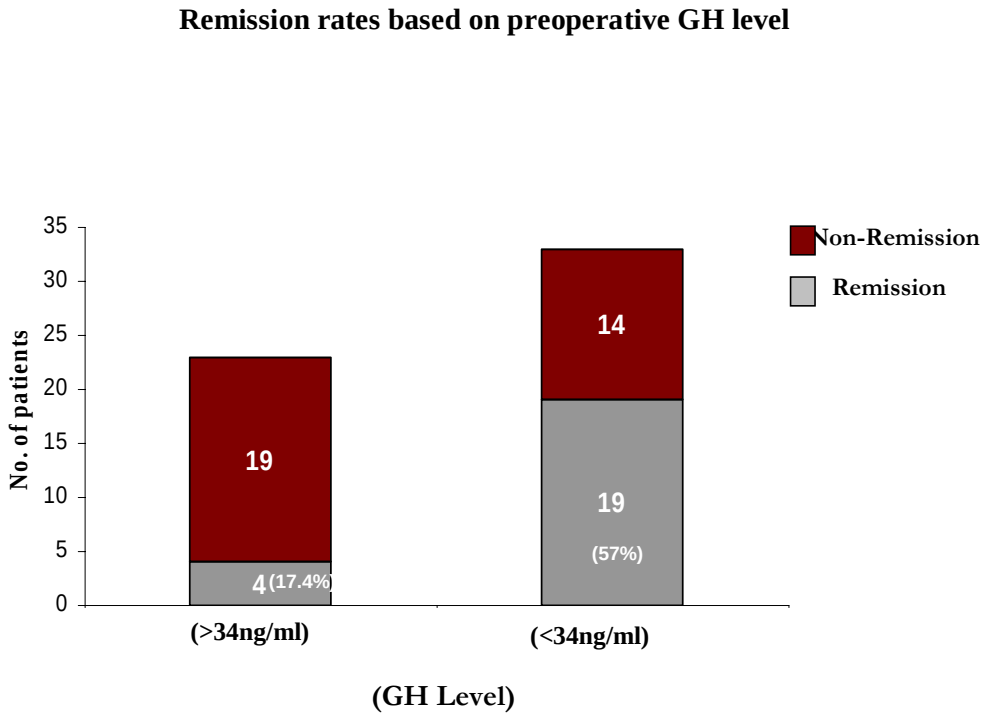

Remission rates based on size
18
16
14
12
10
8
6
4
2
0
2
3
9
5
5
6
9
8
3
1
6
0
Microadenoma
A
B
C
D
E
Non-remission
Remission

Figure 7


Remission rates based on preoperative GH level
35
30
25
20
15
10
5
0
No. of patients
19
4 (17.4%)
14
19
(57%)
Non-Remission
Remission
(>34ng/ml)
(<34ng/ml)
(GH Level)

Remission rates and invasiveness:

In the non-invasive adenomas, the overall cure rate was 44.4% (16 out of 36 patients), whereas in the invasive group, the cure rate was 35% (7 out of 20 patients). The p-value was 0.49.

Remission rates and duration of symptoms:

Based on the duration of symptoms, the patients were divided into 2 groups. Patients with duration of symptoms less than 24 months had an overall cure rate of 41% (7 out of 17 patients). Patients with duration of symptoms more than 24 months had the same cure rate of 41% (16 out of 39 patients). The p-value was 0.99.

Remission rates association with immunostaining characteristics:

Immunostaining for cytokeratin to classify tumours into sparsely and densely granulated variants, was available in 52/56 patients. In the 52 patients, 25 patients had sparsely granulated growth hormone adenomas and the rest of the 27 patients were densely granulated. 6/25 of the patients with sparsely granulated variety were in remission as compared to 8/27 in the densely granulated category. The p-value was 0.64.

Table 4 shows the results of the univariate analysis for each variable for predicting a cure. Included in the analysis are 56 patients who had follow-up GH levels. A univariate analysis was performed using the variables: duration of symptoms, preoperative GH levels, size of tumor, Hardy's stage, invasiveness, extent of excision and whether the tumour was sparsely granulated or not. These were compared with the outcome variables – cure or failure (cure- glucose suppressed level of GH < 1 ng/ml). Duration of symptoms more than 24 months had an Odd's ratio of predicting non-remission of 1.01. Macroadenomas were 2.33 times likely to have non-remission as compared to microadenomas. Invasiveness was found to be not significant in predicting the outcome (Odd's ratio of 0.67). A radical excision of the adenoma had a 2 times greater chance of remission as compared to non-remission. Electron microscopy findings (sparsely granulated adenomas) also were not significant in predicting cure (Odd's ratio of 0.75).

Preoperative growth hormone level of < 34 ng/ml meant that there was a 6.45 times greater possibility of cure as against a level of > 34 ng/ml. (95% CI 1.56-28.78, p-value 0.002).

Similarly, a lower Hardy's grade of tumour (A, B, C) had a 6.61 times of remission as compared to Hardy's grade D or E tumours (95% CI 0.71-153.6, p-value 0.45).

Hence preoperative GH level was the most significant factor associated with a cure.

Table 4

Predictor variables		Outcome variables		Odds ratio	95% CI	P-vALUE
		Cure =23	Failure=33			
Duration of Symptoms	**<24 months**	**7**	**10**	1.01	**0.27-3.7**	0.99
	>24 months	**16**	**23**			
Preoperative GH values	<34ng/ml	19	14	6.45	1.56-27.78	0.002
	>34ng/ml	4	19			
Size of Tumor	Microadenoma	3	2	2.33	0.28-3.38	0.33
	Macroadenoma	20	31			
Hardy's Grade	A,B,C	19	23	6.61	0.71-153.6	0.45
	D,E	1	8			
Invasive-ness	Invasive	7	13	0.67	0.91-2.39	0.49
	Noninvasive	16	20			
Extent of excision	Radical	19	23	2.07	0.48-9.42	0.27
	Sub-total	4	10			
Immuno Histochemistry Sparsely granulated	YES	6	19	0.75	0.18-3.03	0.64
	NO	8	19			

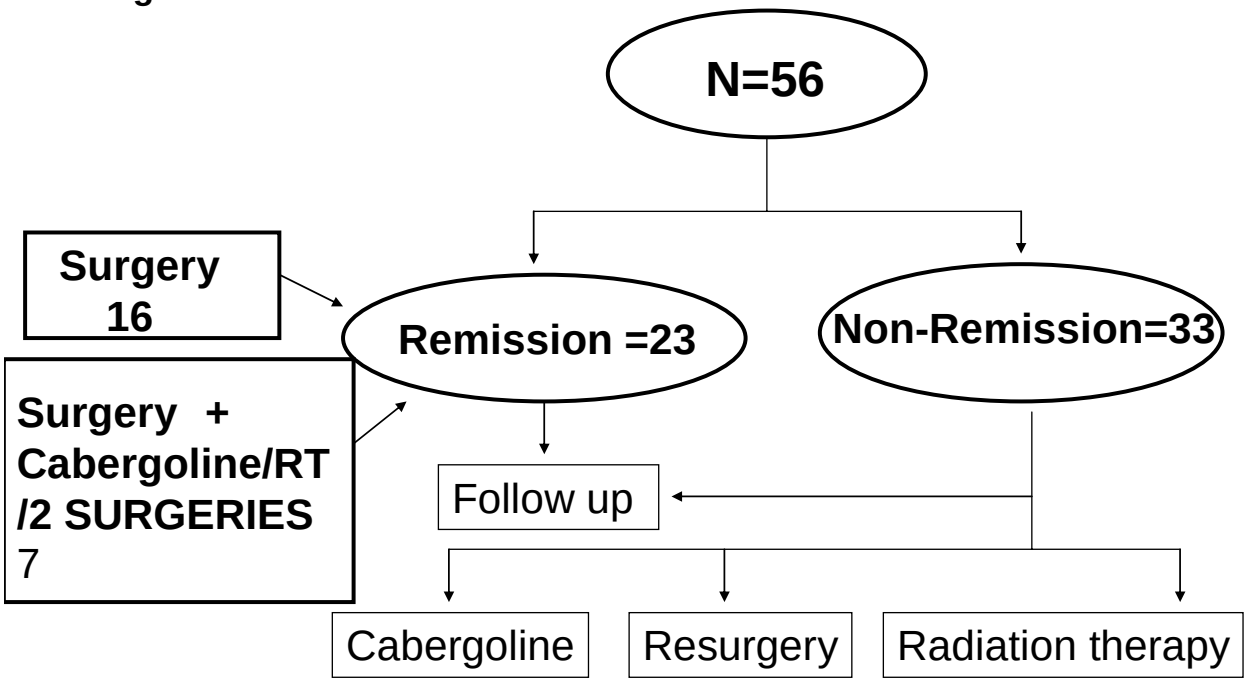
Cure rates and multimodality treatment:

At follow-up, based on nadir post glucose suppression GH levels, sixteen patients (28.5%) were in remission after surgery (mean follow-up 20 months), while 40 (71.5%) were surgical failures. The 40 patients who were in non-remission were kept on close follow-up, started on medical therapy, radiation therapy or underwent second surgery.

Of the fifty six patients, 2 underwent a second surgery and 13 patients received radiation therapy (11-SRT, 2-Conventional). One more patient went into remission after 2 surgeries, one after 2 surgeries and radiation therapy, 2 patients after surgery and radiation therapy and 4 patients with surgery and medical therapy (dopamine agonists). Therefore the final cure rate of initial surgery + 2^{nd} surgery + RT + Medical therapy was 23/56 (41%). A total of 33 patients were still not in remission after multi-modality treatment.

Interestingly, when IGF-1 was used in isolation, as criterion for remission , the cure rates were much higher. IGF-1 was available in 42 of the 56 patients with follow-up. 30 out of the 42 patients were in remission (71.42 %). In this group, there was a subset (8 patients), whose post glucose suppressed serum growth hormone levels were more than 1 ng/ml but less than 5 ng/ml. Post glucose suppressed GH values of < 5 ng/ml have been considered safe enough to lower mortality rates to expected levels (38). Hence, although not adhering to the stringent criteria, these patients with a normalized IGF-1 and GH level between 1-5 ng/ml could be considered to be in partial remission. The various treatment modalities used in the patient management are depicted in Figure 8.

Figure 8.



Remission: Glucose suppressed GH level < 1 ng/ml

(All the values are glucose suppressed GH levels)

CASE REPORTS:

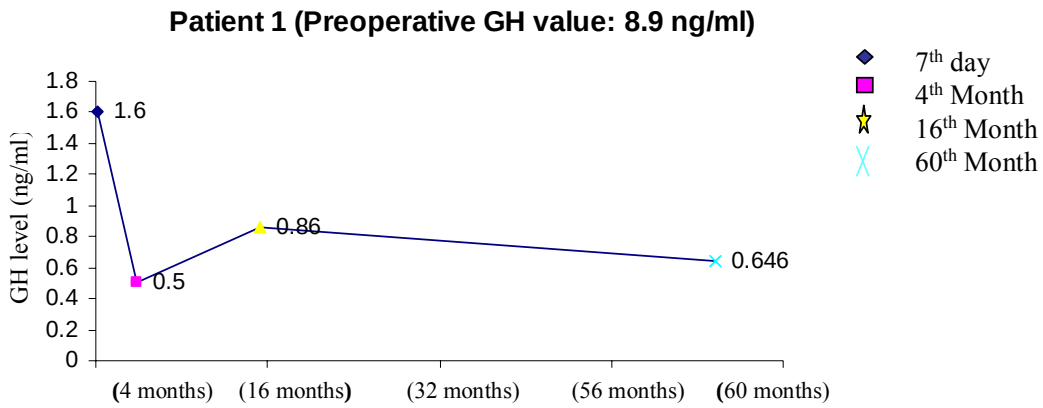
These case reports show the varied response to surgery and the multimodality treatment that is required for cure of this difficult disease.

Case 1: (Figures 9, 10)

This 38 year old lady presented with acromegalic features and investigation revealed a Hardy's grade B pituitary macroadenoma. She was not a diabetic or hypertensive. Vision was normal. She was on Tab. Eltroxine. The preoperative GH level was 8.9 ng/ml. She underwent transsphenoidal radical excision of the tumour. The next day GH was 0.8 ng/ml and the 7^{th} day value was 1.6 ng/ml. At 5 years follow-up, she continues to be in remission, with the GH level of 0.646 ng/ml and normalized IGF-1 level (117 ng/ml). She was still on Tab. Eltroxine.

Fig 9 (Case 1): MRI (T1 coronal with gadolinium) showing a grade B pituitary adenoma

Figure 10



(Post operative follow-ups)

Case 2: (Figures 11, 12)

This 39 year old lady presented with acromegalic features for 3 years. Her vision was normal and she was a hypertensive on medication. MRI showed a Hardy's grade A macroadenoma with invasion of the sphenoid sinus. She was on both Tab. Prednisolone and Tab. Eltroxine as replacements. The preoperative GH level was 11.3 ng/ml. She underwent radical excision of the pituitary adenoma. The histopathology was reported as sparsely granulated GH adenoma. The 1st post operative day GH level was 4.35 and the 7th post operative day GH level was 2.36 ng/ml. Her GH level continued to fall at follow-up but at 22 months, it rose to 3.48 ng/ml. She was started on Tab. Cabergoline and at last follow up (36 months), she was in remission with a GH level of 0.747 ng/ml and an IGF-1 level of 189 ng/ml (normal for age and gender). She continued to be on Tab. Eltroxine, Tab. Amlodipine for blood pressure and Tab. Cabergoline.

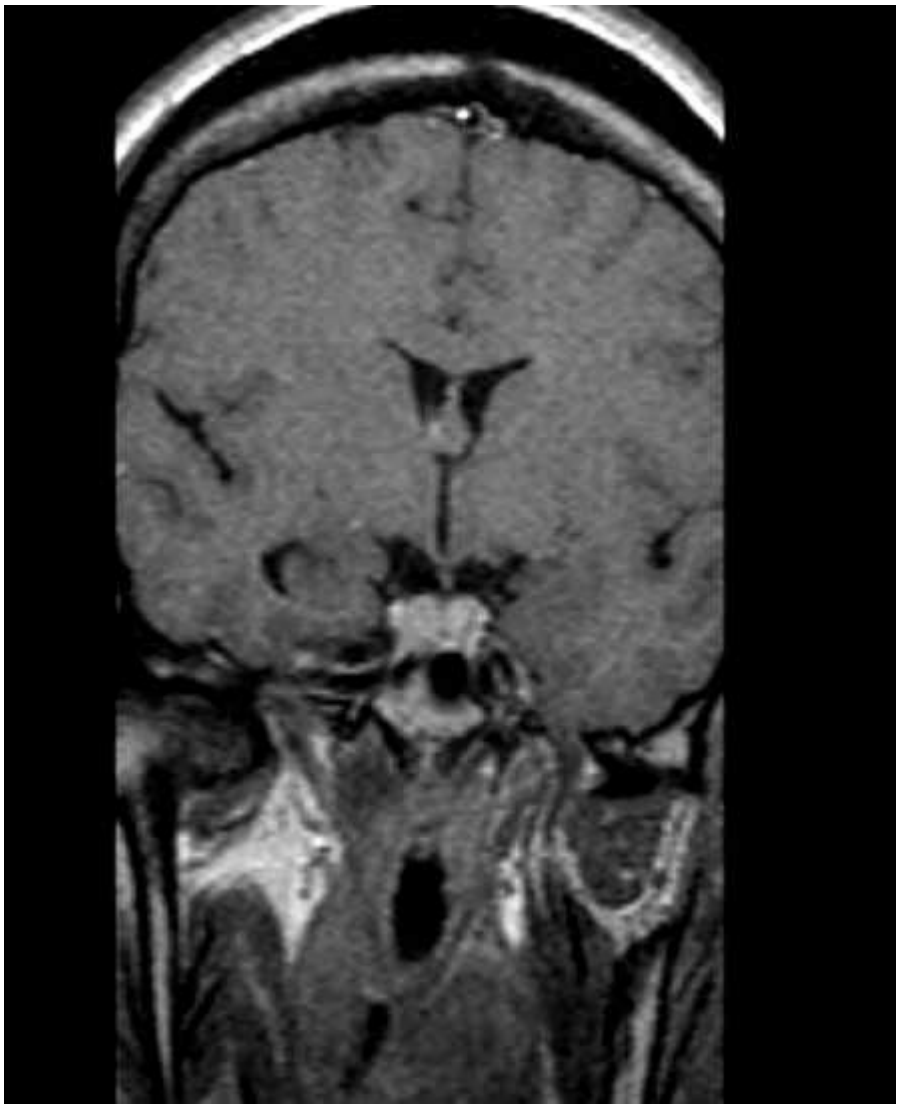
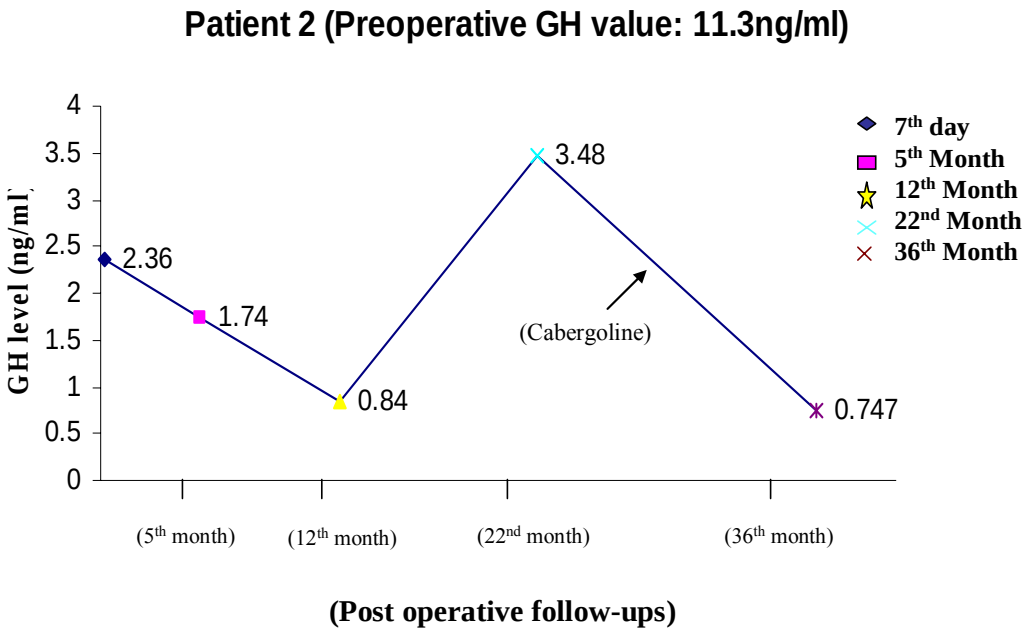


Fig 11(Case 2): MRI (T1 coronal with gadolinium) showing a grade A pituitary macroadenoma with sphenoid sinus invasion.

Figure 12


Patient 2 (Preoperative GH value: 11.3ng/ml)
GH level (ng/ml)
4
3.5
3
2.5
2
1.5
1
0.5
0
2.36
1.74
0.84
3.48
(Cabergoline)
0.747
7th day
5th Month
12th Month
22nd Month
36th Month
(5th month)
(12th month)
(22nd month)
(36th month)
(Post operative follow-ups)

Case 3: (Figures 13, 14, 15)

This 44 year old male presented with acromegalic features. The preoperative GH level was 66.3 ng/ml. MRI showed a microadenoma but there was invasion into the right cavernous sinus. He was a hypertensive on medication. The cortisol and thyroid axes were normal. He underwent radical excision including the portion in the cavernous sinus. The 7^{th} post operative day GH level was 2.1 ng/ml. However his GH level continued to rise at serial follow-ups. MRI brain did not show any residual tumour. He underwent Stereotactic radiation therapy one and half years after surgery. At last follow-up (5 years), his GH level had decreased to 1.3 ng/ml and the IGF-1 was normalized for age and gender (105 ng/ml).

Fig 13(Case 3): MRI (T1 coronal with gadolinium) showing a pituitary microadenoma with invasion of the right cavernous sinus.

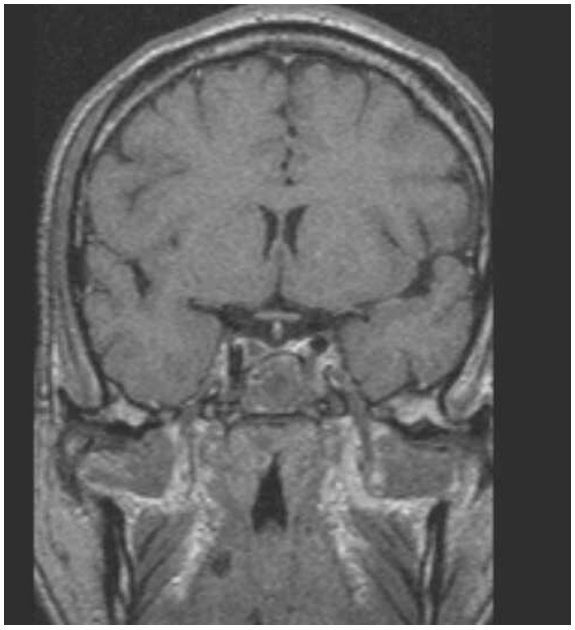
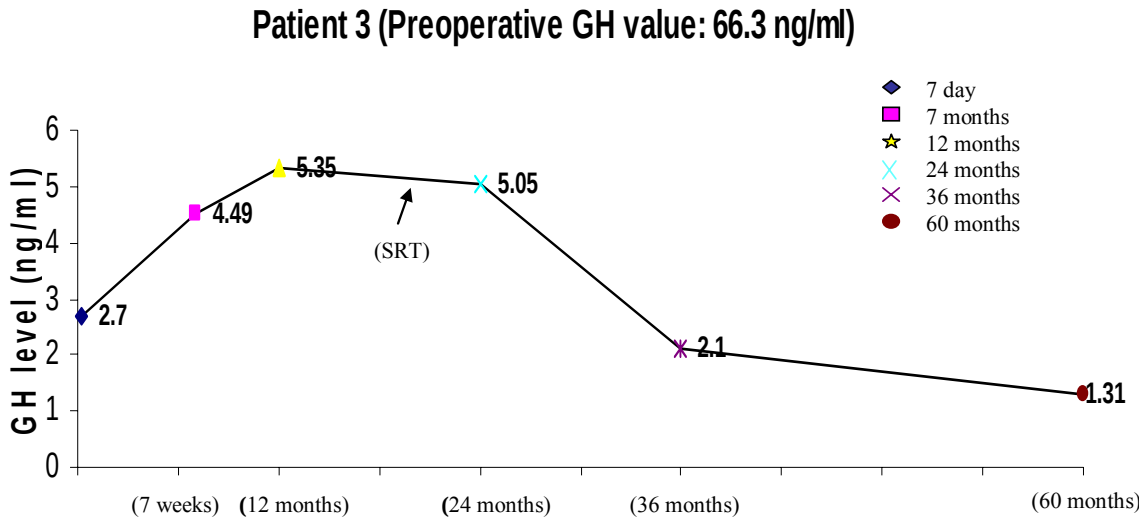


Fig 14(Case 3): Post operative MRI at 8 months following surgery (T1 coronal with gadolinium) not showing any residual tumour.

Figure 15.



(Post operative follow-ups)

Case 4: (Figure 16, 17, 18)

This 17 year old male presented with acromegalic features. His random GH level was 99 ng/ml. MRI showed a Hardy's grade C pituitary adenoma with invasion into the right cavernous sinus. His vision was normal and he was not on replacements. He underwent radical excision of the pituitary adenoma on 15/6/2001. Post operative CT scan showed evidence of residual tumour near the right cavernous sinus. The GH level on the 1st post operative day was 26.1 ng/ml. He was lost to follow-up and came back only after 5 years when the MRI showed a large grade E tumour. He was on Tab. Prednisolone and Tab. Eltroxine. The GH level before the second surgery was 77 ng/ml. He underwent transsphenoidal resurgery on 13/12/2005 and the GH level on the seventh day following resurgery was 9.48 ng/ml. He was advised stereotactic radiation therapy, which he took in September 2006. He was also started on Tab. Cabergoline. His GH level during the time he was on SRT had risen again to 27.7 ng/ml. There were no more follow-ups available for this patient.

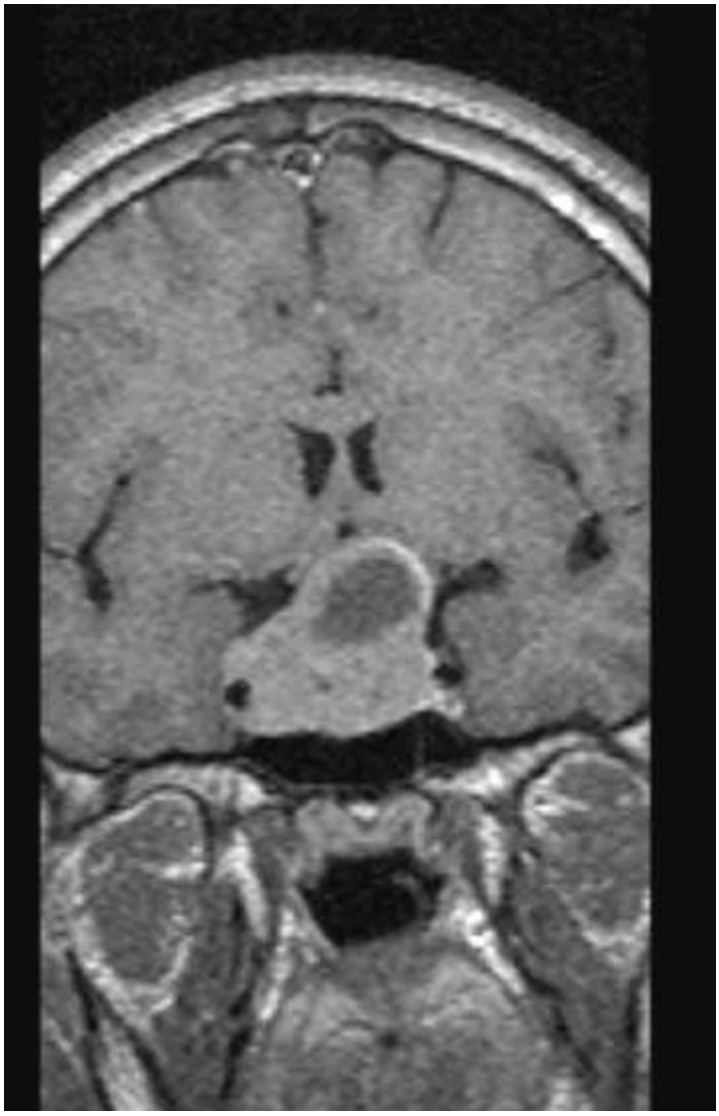


Fig 16(Case 4): MRI (T1 coronal with gadolinium) showing a grade C pituitary macroadenoma with cystic changes and invasion into the right cavernous sinus.

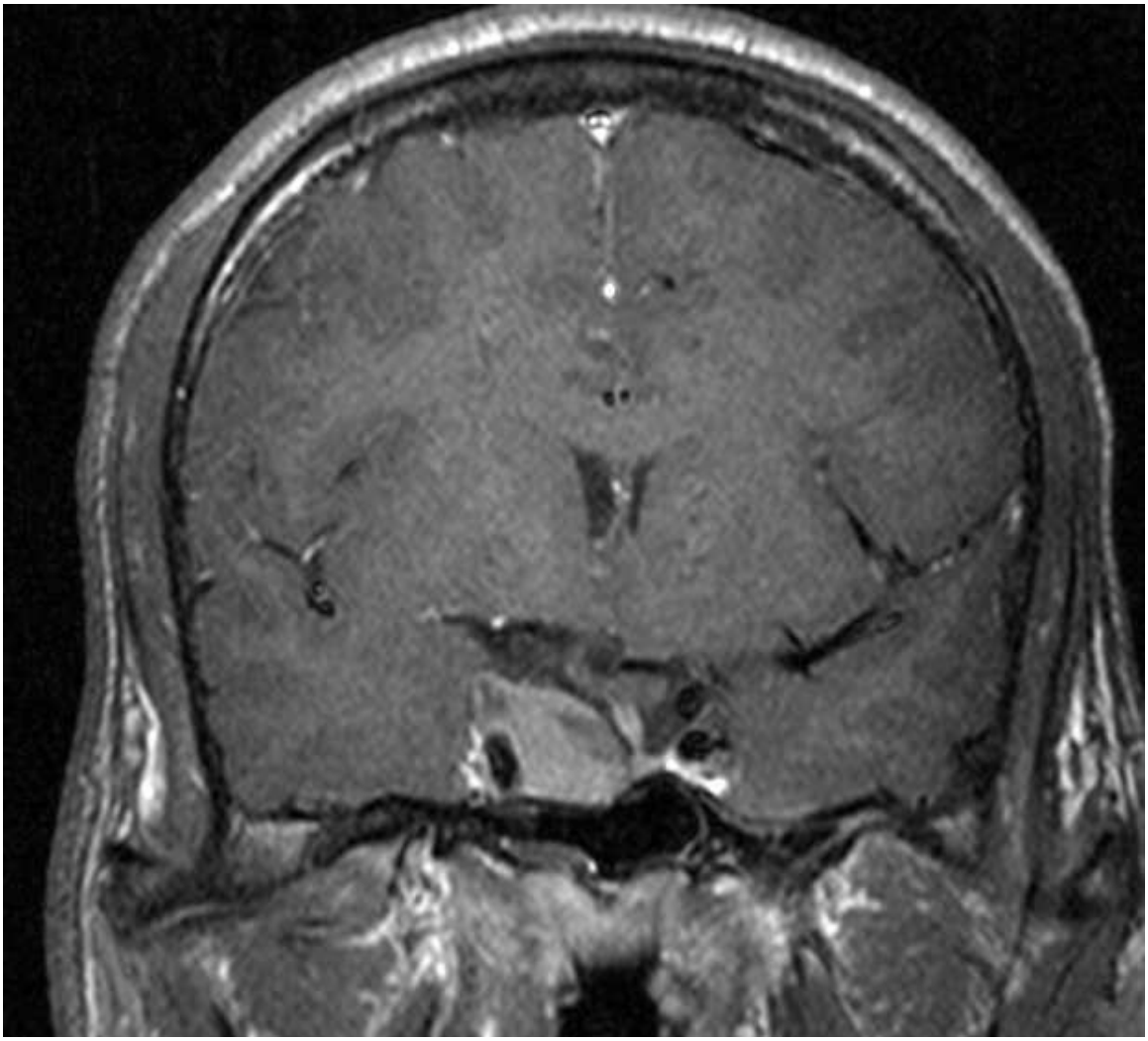
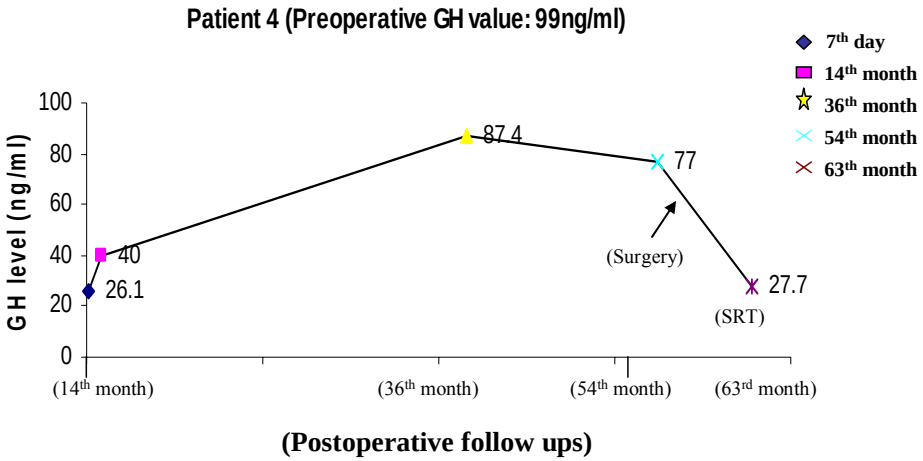


Fig 17(Case 4): Post operative MRI at 54 months (T1 coronal with gadolinium) showing right cavernous sinus residue.

Figure 18


Patient 4 (Preoperative GH value: 99ng/ml)
7th day
14th month
36th month
54th month
63th month
GH level (ng/ml)
100
80
60
40
20
0
87.4
77
40
26.1
27.7
(Surgery)
(SRT)
(14th month)
(36th month)
(54th month)
(63rd month)
(Postoperative follow ups)

Case 5: (Figures 19, 20)

This 40 year old lady presented with acromegalic features for 6 years. Her random GH level was 33.7 ng/ml. MRI showed a 9 mm size microadenoma. Her vision was normal. She underwent radical excision of the pituitary adenoma. The GH level on the 1st post operative day was 0.85 ng/ml and on the 7th post operative day was 1.95 ng/ml. Two follow-ups later at 18 months she continued to be in remission with a GH level of 0.354 ng/ml and IGF-1 of 65 ng/ml. She was a diabetic and hypertensive and she required the same dose of medication as preoperatively.

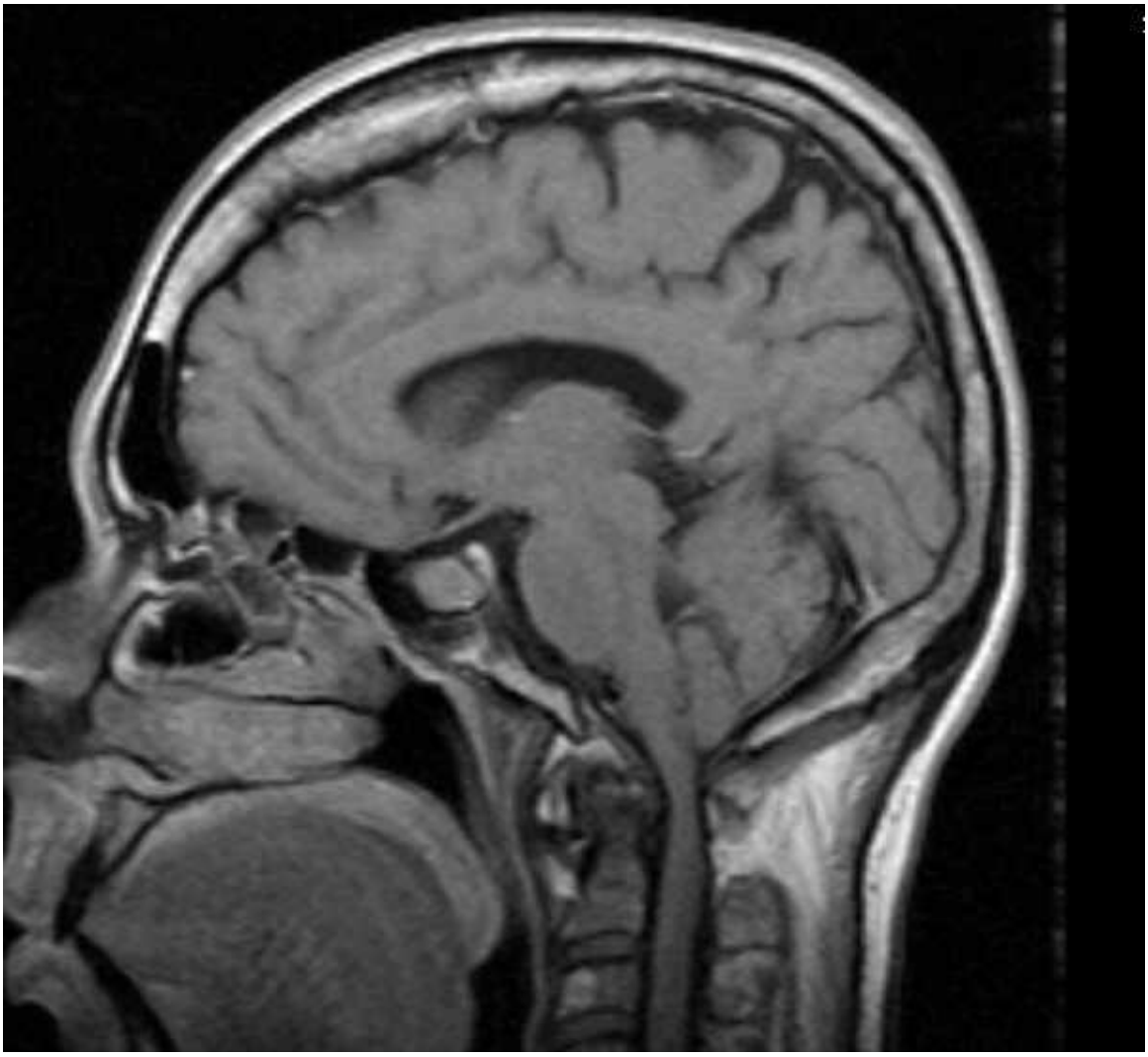
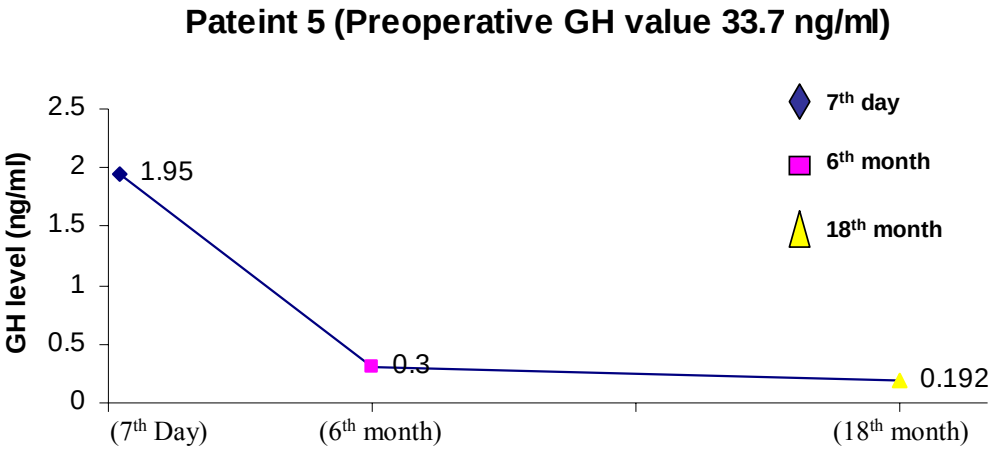


Fig 19(Case 5): MRI (T1 sagittal with gadolinium) showing a pituitary microadenoma.

Figure 20.



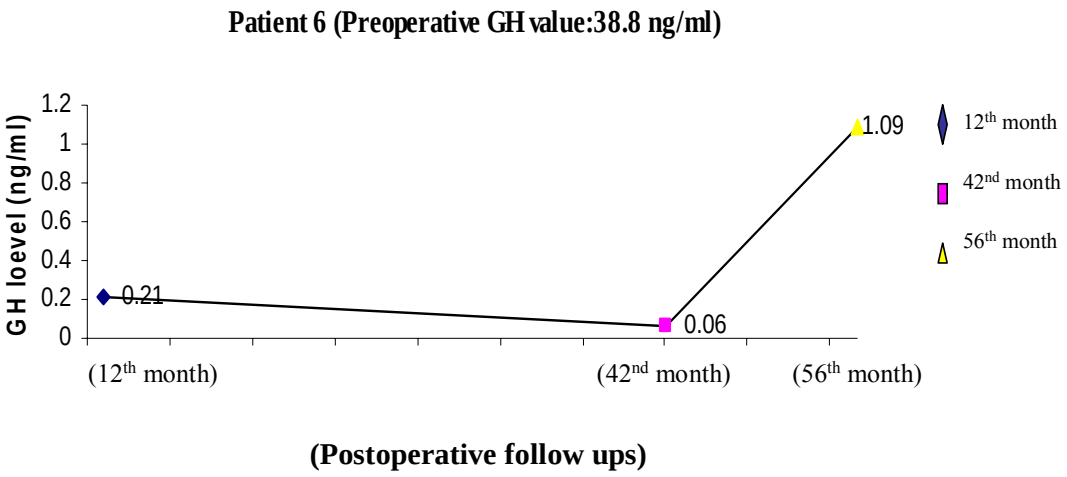
(Postoperative follow ups)

Case 6 : (Figures 21,22)

This 31 year old male presented with acromegalic features for 10 years. The preoperative GH level was 38.8 ng/ml. MRI showed a Hardy's grade A pituitary macroadenoma. . He underwent radical excision of the pituitary adenoma. The 1st post operative day GH level was 0.8 ng/ml. He was in remission till 56 months after surgery when his GH level showed an increase to 1.09 ng/ml. However since the IGF-1 level was normal (70 ng/ml), he is under observation. He would require adjuvant therapy should the IGF-1 level rise.

Fig 21(Case 6): MRI (T1 coronal with gadolinium) showing a grade A pituitary macroadenoma.

Figure 22.


Patient 6 (Preoperative GH value:38.8 ng/ml)
GH loevel (ng/ml)
1.2
1
0.8
0.6
0.4
0.2
0
0.21
0.06
1.09
(12th month)
(42nd month)
(56th month)
12th month
42nd month
56th month
(Postoperative follow ups)

DISCUSSION

The treatment of acromegaly is aimed at reduction of GH levels so as to avoid the harmful systemic effects of raised serum GH level. These include cardiovascular, metabolic and neoplastic changes. Transsphenoidal surgery (TSS) remains the first choice of treatment for acromegaly. It is effective and safe and is able to reverse metabolic and cardiovascular complications related to the disease. However, complete removal is not always possible and adjuvant therapy in the form of dopamine agonists, somatostatin analogs and radiotherapy are often required. Although recent studies show encouraging results with somatostatin analogues and GH receptor antagonists, these drugs are exorbitantly expensive and their use in developing countries is limited.

In our study population, preoperative data such as age and sex distribution, tumor grading, duration of symptoms and preoperative GH concentration were comparable with other studies. Among the presenting complaints, visual deficits were present in a higher percentage of patients (35%) as compared to published literature. A large majority (91.6%) of the adenomas were macroadenomas and more than a third (37.5%) of the adenomas were invasive.

Throughout the 1980s, post-operative GH levels < 5 ng/ml were considered as criteria of biochemical remission of disease, allowing a surgical success in more than 75% of patients in some large series. But epidemiologic studies have shown that mortality rates among treated acromegalic patients are higher than those of the normal population until

GH and IGF-I levels are normalized. This means an age and gender normalized IGF-1 and post glucose suppressed GH levels of less than 1 ng/ml.

The latest stringent criteria recommended by the Acromegaly treatment consensus workshop, were used in this study (normal IGF-1 level and post glucose suppressed GH levels of less than 1 ng/ml). The assay used in our institute was a highly sensitive chemiluminescent immunoassay with a sensitivity of 0.01 ng/ml.

The earliest postoperative timing of assessment of cure was 3 months, which according to literature (102) is the earliest time for assessing both post-glucose suppression nadir GH level and IGF-1 level to determine outcome.

A method that would identify those patients who have not responded to surgery in the early postoperative period itself would allow the early institution of adjuvant therapy to normalize the GH level quickly.

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PREDICTORS OF CURE

The early postoperative GH values were evaluated with regard to sensitivity, specificity and their predictive values for the outcome of the operation. We found that the 1st and 7th postoperative day GH levels were highly predictive of the surgical outcome.

Thus, a 1st postoperative day GH level of more than 2 ng/ml implied that at 3 months, the chance of a cure was only 3.6%. Similarly a 7th postoperative day GH level of more than 2 ng/ml implied a chance of cure of only 11.6% at 3 months. These findings support previous reports on the value of early postoperative GH measurements in determining surgical outcome in the treatment of acromegaly. Feelders et al (102) (GH level at 1 week of <0.5 ng/ml), Freda et al (108) (GH level of < 3 ng/ml in days 2-4 after surgery), Takahashi et al (101) (GH < 1ng/ml at 1 month postoperatively) and Valdemarsson et al (100) (Mean GH level at 1 week of <4.8 ng/ml), all these papers have shown a high specificity and predictive value of early postoperative GH levels. Thus, is our institution, patients with an early postoperative GH level of > 2 ng/ml at kept under close observation with serial follow-up evaluations. A persistent high GH level at follow-up or a rise in GH level with a concordant high IGF-1 level, is an indication for adjuvant therapy. However those patients who have a normal IGF-1 level with a raised GH value are not started on additional therapy unless, the IGF-1 level too rises and crosses the age and gender matched normal range.

We also evaluated other predictors of cure in our study namely the duration of symptoms, size of the tumour, preoperative growth hormone levels, Hardy's grade, invasiveness of the tumour and immunohistochemistry findings. By univariate analysis, preoperative GH levels of less than 34 ng/ml were found to be the most statistically significant in predicting a cure, with an odd's ratio of 6.45 and p-value of 0.002. Although the lower Hardy's grade tumours (A, B, C) had an odd's ratio of 6.61 as compared to Hardy's grade D, E tumours, the p-value was not significant (0.45). Microadenomas also fared much better than macroadenomas with a surgical cure rate of 60% as against 26% for macroadenomas and 15% for invasive tumours. These findings were similar to those already described in literature.

The radicality of the excision was not found to be significant in predicting the outcome. This meant that the surgeon's impression of radicality of excision was not a good indicator of the extent of excision.

Cytokeratin immuostaining characteristic of the tumour was also not found to have statistically significant implication on cure. According to literature, sparsely granulated growth hormone secreting pituitary adenomas (cytokeratin positive) tend to be macroadenomas and more invasive and hence have lower surgical cure rates (27-29). However in our study group there was no difference in the cure rates between sparsely and densely granulated adenomas.

CURE RATES

In spite of there being a consensus statement, there are still studies which used higher GH level definitions as cure. An example is the series published by Abbasioun et al (2006) (47) of 151 patients operated over a period of 23 years. They achieved a cure rate of 94.2% using a cure criterion of 10 ng/ml. According to the author, in developing countries with limited resources and limited modalities of adjuvant therapy, clinical control and the patient "being happy" at follow-up, could be considered good outcomes. This could reflect reluctance on the part of authors to accept an extremely stringent criteria for cure, as the cure rates would then be abysmally low.

We used stringent criteria for cure as defined by Guistina et al (Consensus statement, 2000- post glucose suppression nadir GH value of < 1 ng/ml and a normal IGF1).

In published literature, the cure rates with the stringent criteria of nadir GH levels < 1.0 ng/ml during a GTT vary from 35.5% to 84.4% (48,51,53,56,57,58,60,61,62,64)

In this series, when the post-glucose suppressed values of GH were considered alone as criteria of remission, in microadenomas the cure rate was 60% as compared to 26 % in macroadenomas. The remission rates fell to 0% in Hardy's grade E tumours. The overall cure rates attained with multimodality treatment was 41%. A significant proportion of tumours in our study were invasive at surgery or on imaging (35.7%). In this subset, the overall remission rate was 35%, as compared to noninvasive macroadenomas, where the overall cure rate was 45%.

But we found a significant rise in the overall cure rate to 71% when normalized IGF-1 level was used alone as a criteria of cure. IGF-1 levels were available in only 42 of the

56 patients with follow-up. According to Freda et al (69), as long as IGF-1 normalization is maintained, these patients can be observed without additional therapy. Adjuvant therapy is necessary only when IGF-1 shows a rising trend. Swearingen et al (66) had a 57% surgical cure rate using only normal IGF-1 as criterion for cure.

Cure rates with the stringent criteria of nadir GH levels < 1.0 ng/ml during a GTT vary from 35.5% to 84.4% (Refer Table 2). The cure rate in this series is lower than what is described in literature. However a recently published study showed similar cure rates in the United Kingdom (46) that analyzed results from 22 centers (contributing more than 10 cases in the study period) in the United Kingdom, which do transsphenoidal surgery for acromegaly in the duration form 1975-2004. They used a basal GH < 5ng/ml and/or normal IGF-1 levels, 1 year after surgery, as criteria for cure. There were 2 significant observations from the study. The first and surprising one was that cure rates for acromegaly in U.K. were significantly lower than those reported in literature. The overall cure rate was 39%, it was 56% for microadenomas and 26% for macroadenomas. Microdenoma cure rates ranged from 33-71% and macroadenoma cure rated ranged from 8-56%, between the centers. When they compared cure rates from pre-1985 to that from 2000-2004, another interesting trend was noted. Cure rates for microadenoma improved from 38% to 67% and for macroadenomas from 29% to 38%. This stressed on surgical experience and case load as having positive impact on cure rates. They explained the discrepancy between cure rates in their study and those published in literature, as being partly related to the fact that centers which had good results published while others do

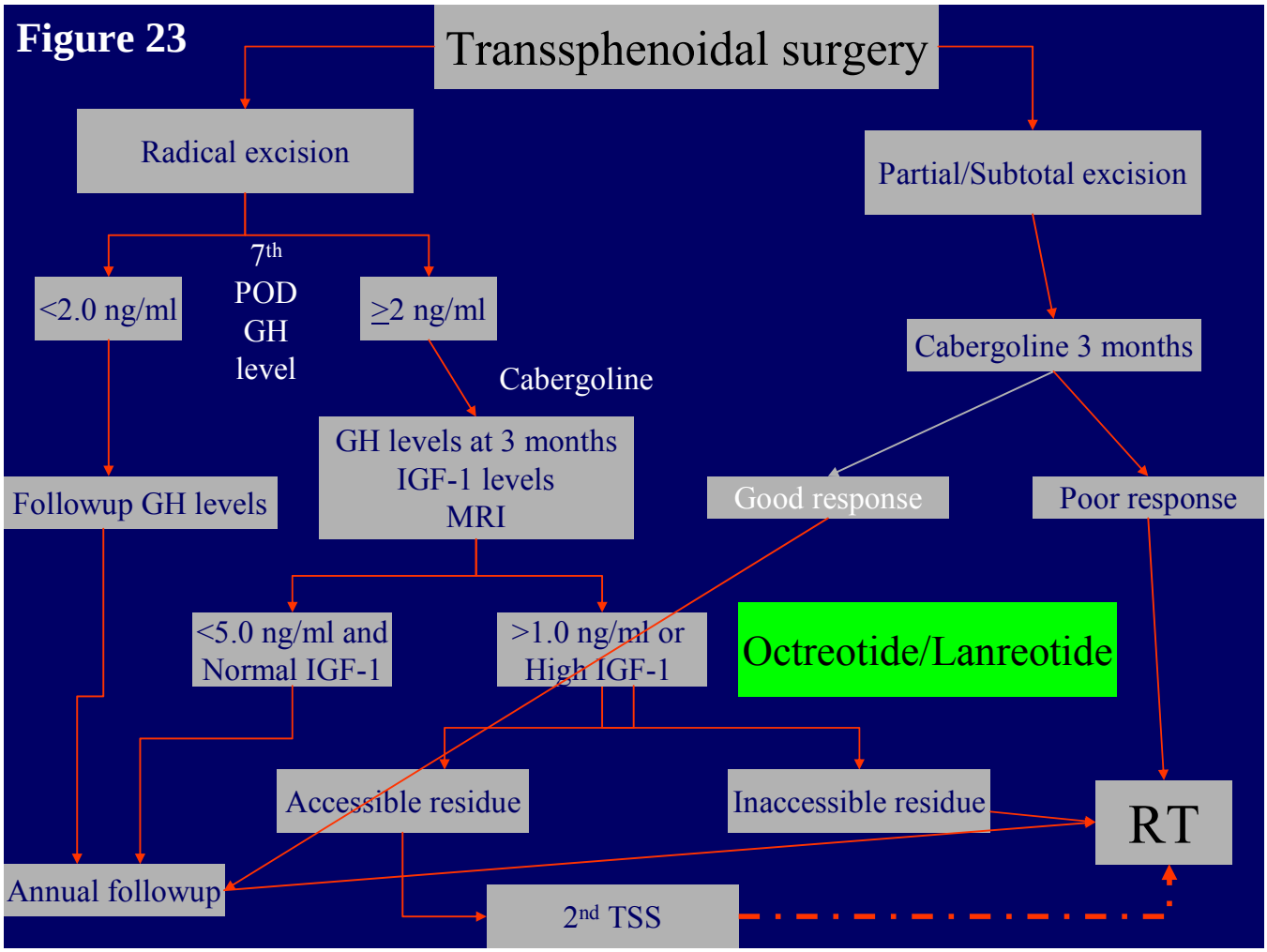
not. This study by Bates et al (46) demonstrates the wide range of cure rates in a developed country, even with less stringent criteria than that recommended by Guistina et al (25). In our study, the overall cure rate was 28.5% using the criteria of post OGTT GH level of < 1 ng/ml, and would be higher if the criterion used by Bates et al (46) is applied. Another reason for the lower cure rates in this study could be the high proportion of invasive adenomas (35%). But other series have also found about 1/3rd of the adenomas to be invasive (62).

Radiation therapy is an important adjunct in the management of acromegaly. In our study group, 13 (23%) underwent radiation therapy, of which 11 patients had stereotactic radiation therapy and 2 underwent conventional radiation therapy. Thus almost a quarter of the patients had adjuvant therapy in the form of radiation. Among these patients, 3 went into remission following RT, the earliest being, 1 year after initiation of radiation therapy. The late effect on decreasing GH levels is a drawback of RT and it has been shown in literature (74), that conventional radiation therapy achieves GH levels of < 1 ng/ml in only 9% of the patients at 2 years and in 77% of the patients at 15 years, when used as an adjunct to surgery. Further, the long term incidence of hypopituitarism can reach upto 90% following radiation therapy (74). Stererotactic radiation therapy achieves the same control of GH levels, with lower incidence of hypopituitarism (5%-29%) (77-79). Therefore patients receiving RT need to be on close follow-up so that hormonal replacements can be started at the earliest evidence of hypopituitarism.

Based on our experience with GH secreting pituitary adenomas, we suggest an algorithm to manage these difficult tumors (Figure 23). Transsphenoidal surgery is the first line of management. In those tumors, where a radical excision has been achieved, further treatment will depend on the 7^{th} postoperative day post glucose suppressed GH levels. As evidenced in our study, those with 7^{th} postoperative day GH level of < 2 ng/ml have a high chance of cure and are hence kept on follow-up without any need for additional treatment. The group of patients who have undergone a radical excision but have a 7^{th} day GH of > 2ng/ml, need to be given a trial of medical therapy with Tab. Cabergoline and the response evaluated after 3 months. Although the criteria of cure is < 1 ng/ml with a normalized IGF-1, literature has shown that those with a normal IGF-1 and GH < 5 ng/ml, have long term mortality rates comparable to normal population and less than 5 ng/ml has been used a criterion of cure (46). Thus, this group of patients can be kept under very close observation with annual check ups. However, in the patients were the IGF-1 level is high and the GH level has not dropped to < 1ng/ml, further management would depend on the MRI Brain findings. If the MRI shows accessible residue, then a second TSS can be planned followed by RT, else direct RT would be the treatment of choice at this juncture.

The patients with partial/subtotal excision of tumors will most likely need RT. But there might be a small subset that might show a good response to Tab. Cabergoline, and these patients can be kept on follow-up.

Figure 23


Transsphenoidal surgery
Radical excision
Partial/Subtotal excision
7th POD GH level
<2.0 ng/ml
≥2 ng/ml
Cabergoline
Cabergoline 3 months
GH levels at 3 months
IGF-1 levels
MRI
Followup GH levels
Good response
Poor response
<5.0 ng/ml and Normal IGF-1
>1.0 ng/ml or High IGF-1
Octreotide/Lanreotide
Accessible residue
Inaccessible residue
RT
Annual followup
2nd TSS

Somatostatin analogs have not been discussed in the algorithm because of the exorbitant costs, which very few of our patients can afford. However, literature does show promising results with this group of drugs, both pre- and post-operatively. These drugs will have a significant role to play in a developing country like ours, once the costs are reduced.

Thus, surgical outcomes for acromegaly remain less than satisfactory and early postoperative GH levels help us in predicting the surgical failures early. With the advent of modern microsurgical techniques including endoscopy-assisted microsurgery operative success for acromegaly may continue to improve. However, for the promising results of newer generation medical therapies in the form of somatostatin analogues and pegvisomant to be translated into reality, the costs need to be reduced so that they can help a larger population.

RECOMMENDATIONS:

1. Primary care physicians play a major role in an early identification of acromegaly and resources should be directed toward increased awareness of the disease and its diagnosis in this provider group. This would lead to early identification of the disease and thus better outcomes.

2. A high preoperative GH level of > 34 ng/ml and Hardy's grade D,E tumours indicate poor outcomes and the inevitable possibility of adjuvant therapy needs to be kept in mind.

3. Early post operative GH levels should be done routinely as they have a good sensitivity and specificity to predict surgical failures.

4. Those patients with a post operative GH > 5 ng/ml and surgically inaccessible tumour residue will probably require adjuvant radiation therapy.

5. Patients with a high GH level (1-5 ng/ml) but a normal IGF-1 need not be started on additional therapy and can be kept on follow-up.

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Appendix-1

PROFORMA FOR PATEINTS UNDERGOING TRASSPHENOIDAL SURGERY FOR ACROMEGALY

Name:

Age/Gender:

Hospital Number:

Date of surgery:

Duration of acromegaly:

Diabetes: yes/no Duration:

Hypertension: yes/no Duration:

Visual symptoms: Acuity/Fields

MRI: Macroadenoma/Microadenoma

Hardy's grade: A/B/C/D/E

Invasive: yes/no

Hemorrhage/Cysts: yes/no

Preoperative Hormonal profile:

Prolactin/Cortisol/TFT/FSH/LH

Basal Growth hormone level:

Growth hormone level (post glucose suppression):

Preoperative replacements: Steroids (yes/no), Eltroxin (yes/no)

Early postoperative growth hormone levels:

First postoperative day:

Seventh postoperative day:

Surgery:

Invasion: nil/left cavernous sinus/right cavernous sinus/sella and sphenoid/all

Excision: partial/subtotal/radical

Gland seen: yes/no

Gland preserved: yes/no

If subtotal resection, site of residue:

Biopsy:

Complications: Meningitis (yes/no), CSF rhinorrhoea (yes/no), hyponatremia(yes/no), hypocortisolism(yes/no).

Radiation therapy: yes/no (SRT/conventional)

Follow-up

Acromegaly:

Diabetes:

Hypertension:

GH levels:

IGF-1 level:

MRI:

Advice:

Appendix- 2

Histopathology methodology

Light microscopy

Routine histology:

Tissues had been fixed in 10% buffered formalin, routinely processed and embedded in paraffin. 5 μm sections were stained with hematoxylin and eosin.

Immunohistochemistry

All tumors were immunostained for the full spectrum of pituitary hormones using the streptavidin-biotin peroxidase complex method. Table 2.1 provides specific information on each of the antibodies used, its dilution and the antigen retrieval method utilized.
As the basic protocol for immunostaining is essentially similar for all antibodies, the protocol given below was followed for each of the antibodies.

Basic protocol:

Immunohistochemical staining was performed by the avidin-peroxidase technique using the appropriate antibody as detailed in Table. Representative 5μ sections of each case were mounted on poly-L-Lysine coated slides and incubated overnight at 37^{0} C. Section of a positive control was used with each batch. Negative controls were achieved by omitting the primary antibody. Sections were deparaffinized followed by rehydration in decreasing ethanol concentrations and placed in distilled water. Appropriate antigen retrieval techniques were adopted depending on the antibody used. The antigen retrieval

technique adopted for each case is specified in the Table. Following antigen retrieval, the sections were covered with normal human pooled serum (1:5 dilution, Institutional Blood Bank) and incubated for 15 minutes. All excess liquid was drained off the slide by gentle tapping and the sections covered with the diluted antibody, and incubated overnight at 4^0 C. The slides were then rinsed in Tris-buffered saline thrice for 5 minutes each. The sections were then drained and covered by diluted secondary antibody, biotinylated rabbit anti mouse (1:200 dilution, DAKO Patts, Denmark) and incubated for 30 minutes at room temperature. The slides were then rinsed in Tris-buffered saline thrice for 5 minutes each. Endogenous peroxidase was blocked with 0.5% hydrogen peroxidase (Qualigens) in methanol by incubating the slides covered with solution for 30 minutes. The sections were again rinsed in Tris-buffered saline thrice for 5 minutes each. The sections were then drained and covered with peroxidase conjugated avidin (1:200 dilution, DAKO Patts, Denmark) and incubated for 30 minutes. The slides were rinsed with 3 changes of Tris-buffered saline for 5 minutes each. The slides were then developed using freshly prepared diaminobenzidine tetrahydrochloride solution (DAKO Patts, Denmark) containing hydrogen peroxide, for 10 minutes. At this point positive controls were checked to ascertain the end of incubation. The sections were counterstained with Harris Hematoxylin for 10 seconds. The sections were then dehydrated, cleared, and mounted with DPX as mounting medium.

Table Specifications of antibodies used for immunohistochemistry

Antibody	Company	Type	Dilution	Antigen Retrieval
Growth Hormone	DAKO	1:80	Monoclonal	Heat
Prolactin	DAKO	1:400	Monoclonal	Heat
ACTH	DAKO	1:150	Monoclonal	No pre-treatment
TSH	BIOGENEX	1:100	Monoclonal	Proteinase K
FSH	BIOGENEX	1:200	Monoclonal	Trypsinisation
LH	BIOGENEX	1:25	Monoclonal	Proteinase K
Alpha subunit	BIOGENEX	1:50	Monoclonal	No pre-treatment

Interpretation of cytoplasmic staining for pituitary hormones, GH, PRL, ACTH, TSH, FSH, LH and α-SU alpha subunit

Tumors with cells displaying dark brown strong cytoplasmic stain were considered immunopositive for the corresponding antibody.

Appendix-3

IGF-1 Adult reference ranges

(IGF-1, ng/ml)

Age (Yr.)	Median	Central 95% Range	0.1%ile
21-25	203	116-358	84
26-30	196	117-329	87
31-35	188	115-307	87
36-40	176	109-284	83
41-45	164	101-267	76
46-50	154	94-252	70
51-55	144	87-238	65
56-60	135	81-225	60
61-65	126	75-212	55
66-70	118	69-200	51
71-75	110	64-188	47
76-80	102	59-177	43
81-85	95	55-166	40